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## Let your HEAD take you

*(The average American today has a choice of just going where "his feet take him", or choosing wisely the course to follow. Let's skip ahead 10 years, and take a look at John Jones—and listen to him . . .)*

"SOMETIMES I feel so good it almost scares me.

"This house—I wouldn't swap a shingle off its roof for any other house on earth. This little valley, with the pond down in the hollow at the back, is the spot I like best in all the world.

"And they're mine. I own 'em. Nobody can take 'em away from me.

"I've got a little money coming in, regularly. Not much—but enough. And I tell you, when you can go to bed every night with nothing on your mind except the fun you're going to have tomorrow—that's as near Heaven as man gets on this earth!

"It wasn't always so.

"Back in '46—that was right after the war and sometimes the going wasn't too easy—I needed cash. Taxes were tough,

and then Ellen got sick. Like almost everybody else, I was buying Bonds through the Payroll Plan—and I figured on cashing some of them in. But sick as she was, it was Ellen who talked me out of it.

"Don't do it, John!" she said. 'Please don't! For the first time in our lives, we're really saving money. It's wonderful to know that every single payday we have *more* money put aside! John, if we can only keep up this saving, think what it can mean! Maybe someday you won't have to work. Maybe we can own a home. And oh, how good it would feel to know that we need never worry about money when we're old!"

"Well, even after she got better, I stayed away from the weekly poker game—quit dropping a little cash at the hot spots now and then—gave up some of the things a man feels he has a right to. We didn't have as much fun for a while but we paid our taxes and the doctor and—we didn't touch the Bonds.

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**THE JOURNAL OF NEUROPHYSIOLOGY**

# DORSAL ROOT POTENTIALS OF THE SPINAL CORD

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THOUGH catelectronic potentials in the dorsal roots of the spinal cord were first observed by Gotch and Horsley in 1891, it was not until recently that they were systematically investigated (1, 2, 4, 5, 6, 9, 10, 11, 18). The present paper reports an extension of this investigation which particularly concerns an experimental analysis directed towards the elucidation of the nature and mode of production of the dorsal root potentials (henceforth called d.r.p.). A preliminary account has already been published (13).

The present investigation has been restricted almost entirely to the isolated oxygenated spinal cord of the frog—some 30 experiments in all.\* The technique has recently been described (14). With the usual disposition of the recording electrodes, the grid lead has been on the isolated cut end of the dorsal root, and the earthed lead has been a fine platinum loop surrounding the dorsal root as it emerges from the spinal cord (cf. inset diagram, Fig. 1). Some of the experiments were performed on "spring" frogs in Sydney at temperatures of 20 to 24°C. (cf. 13), the others in Dunedin on "winter" frogs at temperatures of 13 to 16°C. The latter gave much slower responses.

## *A. Time course of dorsal root potential*

1. *Initiated by dorsal root volleys.* The d.r.p. set up by the application of a very weak shock to an adjacent dorsal root rises to a rounded summit and the subsequent decay is approximately exponential in its latter part (Fig. 1a). The respective values for latent period, time to summit and half-time of the exponential decay are usually within the following limits: 3–5, 40–80, and 30–80 msec. The longer values have been given by winter frogs, the shorter by spring frogs.

As the stimulus strength is increased (Fig. 1b), not only does the d.r.p. become larger, but also its summit moves earlier (to about 20 to 40 msec.), and eventually it no longer decays exponentially to its original base line. As shown in Figure 1c and d a prolonged negative potential has appeared, and the d.r.p. can usually be seen to decay to the temporary base line of this prolonged negative potential with much the same time course as before. In some preparations the slowed decay is not thus sharply separable into two phases. Finally, a very large dorsal root volley sets up a d.r.p. rising steeply to a relatively flat top (Fig. 1d), and the residual slow potential may be as much as 30 per cent of the summit height. The steep rising phase is inter-

\* It is to be noted that the potentials recorded in 1933 by Umrath (29) and Umrath (28) must largely have been from the dorsal roots. The earth was indifferently placed on the body of the frog. For example, in all figures in Umrath's paper (29) the prolonged downward deflection appears to be produced by a negative catelectrotonic potential of the dorsal roots at their origin from the cord, for that would be the effective location of the earthed lead.

rupted by the spike potential of impulses passing outwards along the dorsal root fibres (cf. 26, 27; Section H below). If the earth lead (E in Fig. 1) makes contact with the cord adjacent to the root, rather than with the root itself, the d.r.p. begins with a brief positive dip (cf. Figs. 6a and b, 7a), which is due presumably to the inverse recording that results from the current flow in a volume conductor (the spinal cord).

2. *Initiated by ventral root volleys.* The following observations on the d.r.p. set up in a dorsal root by an antidromic volley in an adjacent ventral root (Fig. 2) are partly additional to and partly confirm the original descrip-

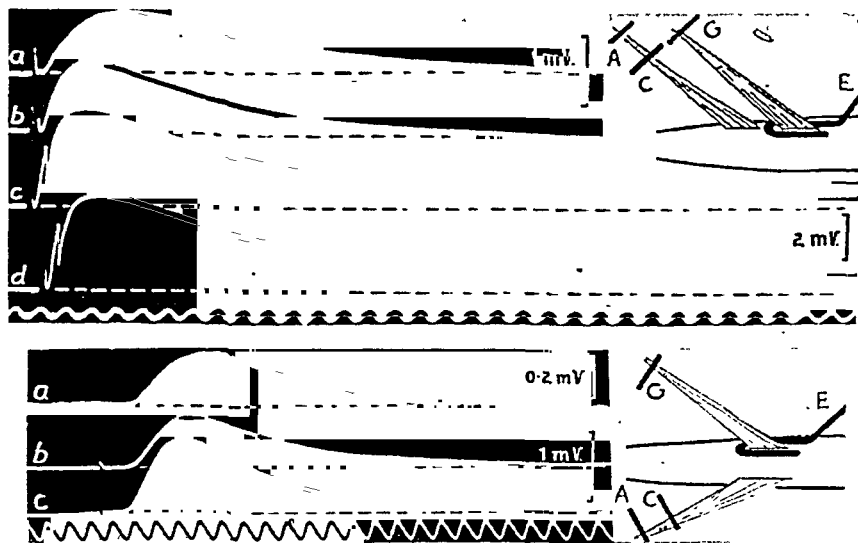


FIG. 1. Dorsal root potentials set up in response to a single stimulus applied to a dorsal root. Stimulus strength progressively increased from *a* to *d*. Higher amplification for *a*. Base lines drawn in for all records. Time: 1 d.v. 10 msec. Temperature: 23.5°C. Disposition of electrodes shown in inset diagram, E earth, G grid, C cathode, A anode.

FIG. 2. Dorsal root potentials in 10th dorsal root (D10) set up in response to a single stimulus applied to the ventral root (V10). Stimulus strength increased from *a* to *c*. Higher amplification for *a*. Base lines and time as in Fig. 1. Temperature: 20°C. Disposition of electrodes shown in inset, E earth, G grid, C cathode, A anode.

tion of Barron and Matthews (2). Similar potentials were recorded inversely by Umrath (29, Fig. 1a, 2a and c, etc.).

(i) Latent period: There may be an initial slowly rising negativity, but the main potential has a long latent period (10 to 15 msec.).

(ii) The potential rises to a rounded summit of usually no more than 1.0 mV. in about 40 to 70 msec. With small antidromic volleys the summit is a little later (Fig. 2a and b), but the relative slowing (never more than 50 per cent) is less than with the d.r.p.'s set up by dorsal root volleys.

(iii) The latter part of the decay is approximately exponential, with half-times (25–70 msec. in different preparations) closely resembling those

obtained for the d.r.p.'s set up in the same root by small dorsal root volleys. The decay of the d.r.p. to the base line is always exponential even with the largest antidromic volleys, no prolonged potential ever being observed.

### B. Electrotonic spread of d.r.p.

Barron and Matthews (2) observed that the d.r.p. spreads decrementally along the dorsal root just as an electrotonus would, *i.e.*, with an exponential

FIG. 3 *a*. Dorsal root potentials set up by a maximum dorsal root volley (D9) and recorded on D10 at distances from the spinal cord in order from above down of 0, 1.6, 2.7, 3.9, 5.1 mm. Arrows marking the summits show the progressive delay with increasing distance. Temperature: 13°C. *b*. Log. of summit height (mV.) of d.r.p. plotted as ordinates against distance from spinal cord at which recorded as abscissae. Open circles for series of Fig. 3*a*; solid circles for simultaneous series of d.r.p.s set up by maximum ventral root volley (V10).

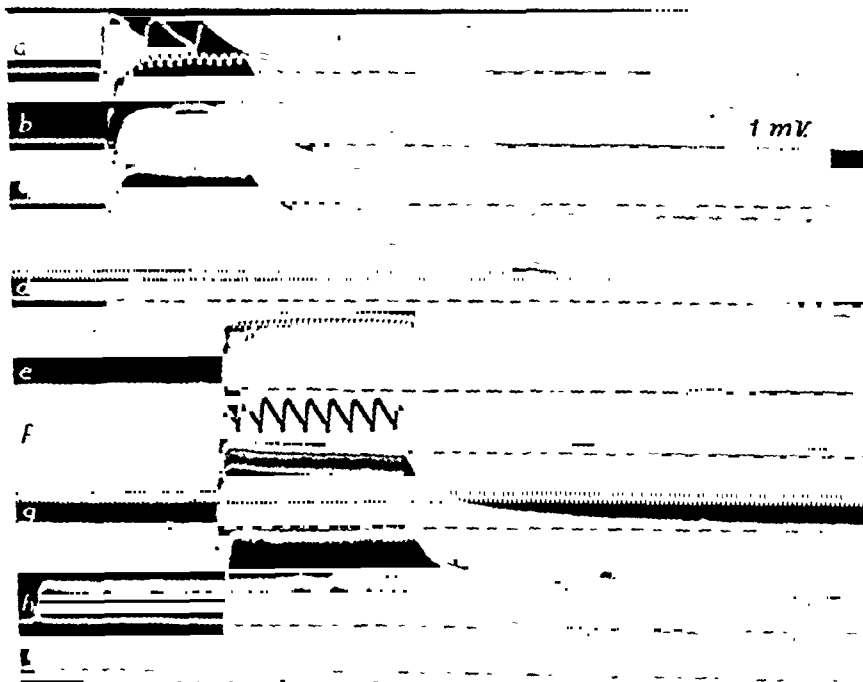
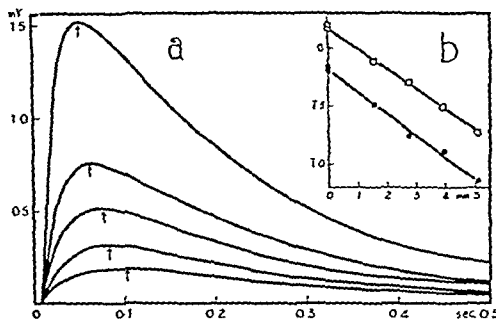


FIG. 4. D.r.p.'s set up in D9 by repetitive stimulation of D10. *a*, *b*, and *c*, submaximum stimulation at 9, 50 and [200/sec.]; *d*, stimulus strength increased 8-fold, [200/sec.] Temp.: 20°C. Time: 1 d.v. = 10 msec. *e*, *f*, and *g*, another experiment at 100, 25 and [200/sec.] submaximum stimulation; *h* and *i*, at [200/second] maximum stimulation. Temp.: 22.5°C. Time: 1 d.v. = 10 msec. Same potential scale throughout.

decay. We have confirmed this for the d.r.p.'s set up both by dorsal and ventral root volleys, halving occurring in every 1.5 to 1.7 mm. (Fig. 3a and b), which is in close agreement with their value of 1.7 mm. Further, the time courses of the d.r.p.'s show a progressive slowing the further the propagation (Fig. 3a), just as would be expected for electrotonically transmitted potentials.

### C. Summation of d.r.p.'s

In general we have confirmed the findings of Barron and Matthews (2) in regard to summation of d.r.p.'s set up by two volleys (Fig. 6a) or by repetitive volleys (Fig. 4), but our observations also give additional evidence for



FIG. 5. D.r.p.'s set up in D10 by two maximum ventral root volleys (V10) at various intervals apart. Top record shows d.r.p. set up by second volley alone. Time: 1 d.v.  $\approx$  10 msec. Temp.: 20°C.

the above subdivision of the d.r.p. into two phases, the one rapidly decaying, the other slowly decaying.

A second dorsal root volley at a short interval adds but little potential to the first, but it may give a considerable increase in the prolonged potential. This effect is still more obvious with rapid repetitive stimulation. The d.r.p. sums to a plateau but little higher than the single response, but there is usually a large summation of the slowly decaying potential. Thus with weak repetitive stimulation the d.r.p. shows an exponential decay to the initial base line resembling that observed for the single response (Fig. 4a, b, c, e, f, and g). With strong stimulation, however, there is always a large slowly decaying potential, which is usually clearly separated from the initial rapidly decaying phase (Fig. 4d and h). Increase in the rapidity and/or duration of the repetitive stimulation also serves to accentuate the slowly decaying potential (Fig. 4i; cf. 2, Fig. 6E).

Two small ventral root volleys at a short interval may set up a d.r.p. greater than the sum of the d.r.p.'s set up by either volley alone, but with large volleys there is occlusion just as is observed with dorsal root volleys (Fig. 5). There is also occlusion between the d.r.p.'s set up by ventral and

dorsal root volleys. Repetitive ventral root volleys fail to sustain a plateau of d.r.p., and there is never any sign of a slowly decaying potential.

#### *D. Action of nembutal on d.r.p.*

The narcotic nembutal has been applied by soaking the isolated spinal cord for at least 30 minutes in oxygenated Ringer's solution containing the desired concentration. The excess fluid is then drained off and the observations are made with the cord set up in oxygen as heretofore. The effects catalogued below have been uniformly observed in each of the twelve experiments.

(i) The decay of the d.r.p. is greatly slowed, the effect being greater, the higher the concentration. With the deep narcosis given by a concentration of at least 1 in 7,000 nembutal, the decay of the d.r.p. set up even by large dorsal root volleys closely approximates to the exponential form (Fig. 6b), and in any preparation the half-times for the decay of the d.r.p.'s set up by dorsal and ventral root volleys of any size are practically identical. A concentration of 1 in 7,000 nembutal gives half-times for decay ranging between 0.4 to 0.7 sec. in different preparations; 1 in 10,000, 0.3 to 0.5 sec.; and 1 in 20,000, 0.2 to 0.3 sec.

(ii) With deep narcosis the d.r.p.'s set up by all sizes of dorsal root volleys come to have practically identical rising phases (Fig. 7a). As shown in Figure 8, this is largely brought about by a shortening of the normally long rising phases of the d.r.p.'s set up by small volleys.

(iii) As shown in Figure 6d, nembutal has practically no action on the rising phase of the d.r.p.'s set up by ventral root volleys.

(iv) The height of the d.r.p. is not diminished by light narcosis, but 1/5000 nembutal always depresses the d.r.p. set up by dorsal root volleys and abolishes that set up by ventral root volleys.

(v) There is an occlusion even larger than normal between the potentials set up by successive volleys, and the greatly slowed decay causes this occlusion to occur even with long intervals between stimuli (compare Fig. 6b with 6a). Figure 6c shows that the potential set up by strong and rapid repetitive stimulation decays with much the same time course as potentials set up by slow rates of stimulation or even by a small single volley (Fig. 6b). There is no sign of the large addition of slowly decaying potential that is observed normally.

#### *E. Action of convulsant drugs*

Strychnine, curarine and veratrine act similarly in causing a great increase and prolongation (after-discharge) of the discharge of motoneurons in response to a dorsal root volley. In higher doses this discharge occurs in the absence of stimulation. All three drugs greatly prolonged the d.r.p. set up by a dorsal root volley, and it often shows subsidiary humps on the declining phase. Such responses have been illustrated for strychnine by Um-rath (29, Fig. 2b, c and d), Dun (10) and Dun and Feng (11). The time course of the d.r.p. set up by a ventral root volley is not appreciably altered by strychnine 1 in 200,000 (cf. 2, Fig. 2a and c) or veratrine 1 in 100,000, but its potential is considerably reduced, and it is abolished by 1 in 100,000 strychnine.

#### *F. Nature of dorsal root potential*

The following evidence indicates that the d.r.p. resembles the catelectrotonic junctional potentials (endplate potential and synaptic potential) in that the latter part of its decay has a passive electrotonic character.



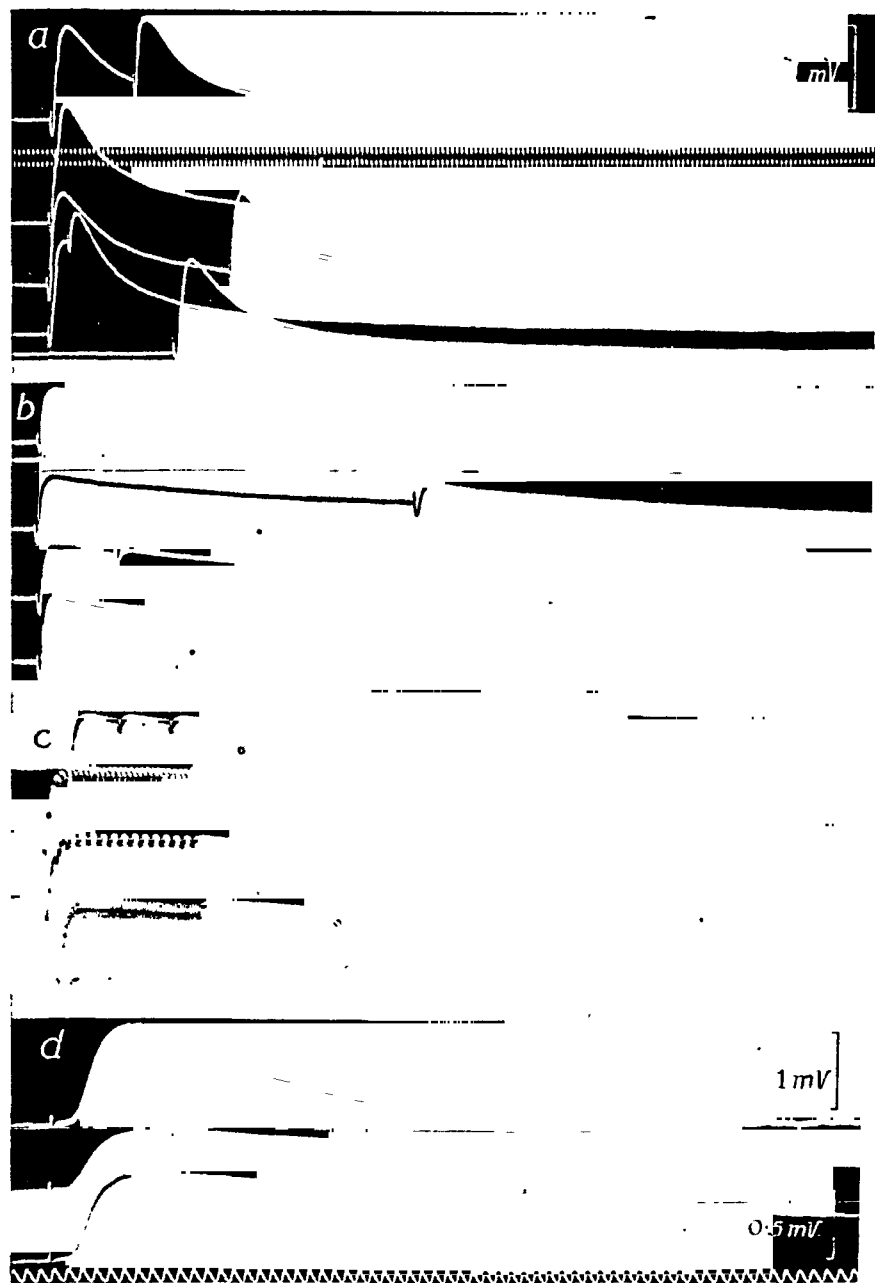


FIG. 6a. D.r.p.'s set up in D9 by two volleys in D10 at intervals of 174, 14, 388 and 43 msec. respectively; lowest record, second volley alone. Potential scale and times shown for *a*, *b* and *c*. Temp.: 20°C.

*b*. Similar series of d.r.p.'s in same experiment, but after soaking cord in 1 in 7,000

1. The d.r.p. is decrementally propagated along the dorsal root just as a catelectrotonus would be, and shows the expected alteration in time-course (section B).

2. The d.r.p. set up by a ventral root volley always decays exponentially in its latter part. An exponential decay with a similar half-time is also observed with the d.r.p.'s set up by small dorsal root volleys (section A).

3. The selective action of narcosis (section D) in affecting to a minor extent the rising phase, and in greatly slowing the time course of the exponential decay of the d.r.p. (whether initiated by a dorsal or a ventral root volley) is most simply explained as being due to a lengthening of the electric time constant of the surface membrane of the dorsal root fibres by the narcotic (cf. section J). This selectivity is particularly evident with the d.r.p.'s set up by ventral root volleys (cf. Fig. 6d).

The following evidence indicates that prolonged activity of internuncial neurones is responsible for the slowly decaying fraction of the d.r.p. which is set up in a normal cord by dorsal root volleys when large and/or repetitive.

(i) Prolonged internuncial activity (after-discharge) is revealed by simultaneous observations on the electrical activity of the motoneurones, as recorded at the ventral root origin. Prolonged synaptic potentials and discharges of impulses are observed (cf. 2, Figs. 11 and 12; 14, Fig. 2b). The slowly decaying fraction of the d.r.p. and this prolonged activity of motoneurones are similarly increased by any augmentation in the intensity of central stimulation provided by the dorsal root volleys, e.g., by increased size of volleys or by an increased frequency or duration of repetitive dorsal root stimulation (section C).

(ii) The electrical activity of the motoneurones further shows that progressive deepening of narcosis shortens and diminishes the internuncial after-discharge. *Pari passu* there is an approximation of the time courses of decay of the d.r.p.'s set up by all intensities of dorsal root stimulation (single or repetitive). Finally, deep narcosis abolishes internuncial after-discharge as well as the differences in decay of the d.r.p.'s (section D.)

(iii) Conversely the convulsant drugs, strychnine, veratrine and curarine, increase and prolong both internuncial after-discharge and the slowly decaying fraction of the d.r.p. (section E). The late humps then often present in the d.r.p. suggest the action of large bursts of internuncial discharges, and such effects are also observed on the motoneurones.

On the other hand the d.r.p. set up by a ventral root volley never gives any evidence with these three tests that it is prolonged by internuncial after-discharge. Thus a more reliable time-constant for the passive process of decay can be calculated from its decaying phase than from the d.r.p. set up by a dorsal root volley. In some preparations the dorsal root volley has to be made very small before the prolonged internuncial activity is shown to be insignificant by two criteria based on the above observations: the half-time of decay shortening to the value found for the d.r.p. set up by a ventral root volley; the short-lasting synaptic potential of the motoneurones (cf. 14, Fig. 5).

On the basis of the time-constant for passive decay it is possible to calculate the time course of the actively depolarizing agent setting up the d.r.p.

---

nembutal for one hour. Top record single response with base line below; then respective volley intervals 820, 170 and 7 msec.

c. Repetitive series taken immediately after (b) at frequencies of 9, 100, 50 and 200 per. sec. Control series before nembutalization shown in Fig. 4a, b, c and d.

d. From above downwards d.r.p.'s set up in D10 by a maximum volley in V10 normally and after soaking for 45 mins. in 1 in 10,000 and 1 in 7,000 nembutal respectively. Upper potential scale for first two records. Temp.: 13°C. Time: 1 d.v. = 10 msec.

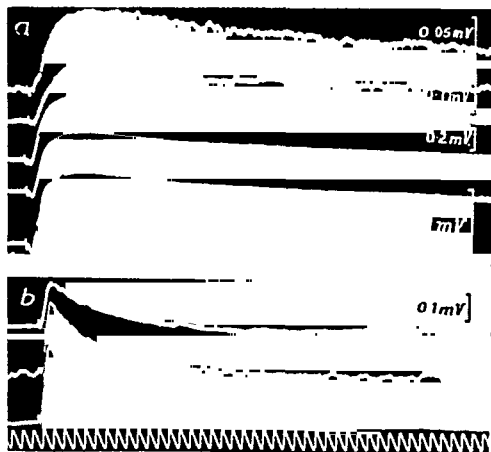


FIG. 7 *a*. D.r.p.'s set up in D9 in response to single stimuli of various strengths to D10. Cord previously soaked for 1 hour in 1 in 5,000 nembutal. Progressively increasing strengths from above down. Lower potential scale for two lower records, respective potential scales shown for top 3 records. Time shown below, *b*.

*b*. Same experiment and conditions as for (*a*). Synaptic potentials set up in V9 in response to single stimuli of various strengths to D9 and D10 together. Potential scale shown for 2 upper records; lowest record has lowest potential scale of (*a*). Time: 1 d.v. = 10 msec. Temp.: 14°C.

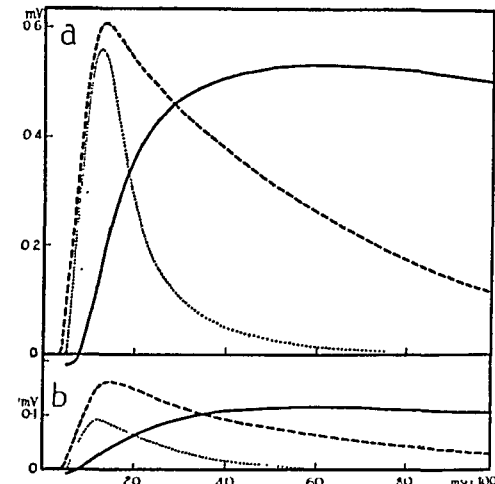
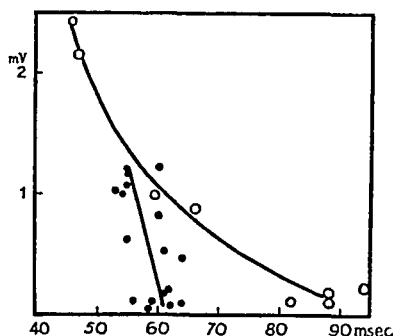


FIG. 9 *a*. Same experiment as Fig. 7. Continuous line shows d.r.p. set up by a large d.r.p. (3rd record of Fig. 7*a*). Broken line shows with same potential scale synaptic potential in ventral root (average of two similar records, one being 3rd record of Fig. 7*b*). Dotted line shows calculated course of active depolarizing agent setting up the d.r.p.

*b*. As for (*a*), but for responses set up by much smaller volleys.

FIG. 8. Same experiment as Fig. 7. Sizes of d.r.p.'s set up by single volley of various strengths plotted as ordinates against the respective times to summits as abscissae. Open circles before nembutal, solid circles after soaking for 1 hour in 1 in 5,000 nembutal—part of series shown in Fig. 7*a*.

(cf. the similar analyses for the endplate potential and the synaptic potential). Since in deep narcosis the time course of the rising phase of the d.r.p. is practically the same for all strengths of dorsal root volley (Fig. 7*a*), the corresponding actively depolarizing agents will have almost identical courses (dotted curves of Fig. 9*a* and *b*), *i.e.*, the time course is independent of the number of nerve impulses acting simultaneously; hence presumably Figure

9 gives the time course for the depolarizing action of a single impulse (cf. the unitary action of Fessard and Matthews, 18).

It must, however, be realized that the d.r.p. has suffered some distortion during electrotonic propagation from the central locus of its origin along the dorsal root fibres to the surface of the cord. The time course of the depolarizing agent would consequently be quicker than shown in Figure 9.

In light narcosis or in the absence of narcosis, further complications are introduced which render any attempt to determine the time course of the actively depolarizing agent of doubtful significance. (i) The rising phase of the d.r.p. set up by a large volley is interrupted by the dorsal root reflex (section H). (ii) The flattened top of large d.r.p.'s (as compared with smaller d.r.p.'s) suggests that a ceiling is being reached in the amount of depolarization that can be produced. Under such conditions it could no longer be assumed (as above) that the rate of depolarization is proportional to the intensity of the depolarizing agent. (iii) The later summit of the d.r.p.'s set up by small dorsal root volleys also suggests some complicating distortion (see section J). With the d.r.p.'s set up by ventral root volleys only complication (iii) plays a significant role. The slower time course of the small d.r.p.'s (Fig. 2) is observed even in relatively deep narcosis. From Figure 6d it can be seen that the narcotic has a negligible effect on the time course of the actively depolarizing agent.

#### *G. Locus at which d.r.p. is produced*

The above concept that the d.r.p. is produced by an actively depolarizing agent acting on a polarizable membrane with a specific electric time constant raises the question: on what structure is this action exerted? As Barron and Matthews point out, the specific transmission of the d.r.p. outwards from the cord along dorsal root fibres strongly suggests that it is produced in the central pathway of these fibres. It has been shown that a catelectrotonic potential of a membrane is abolished by the passage of a propagated impulse (22). It is therefore important to investigate the action which a volley fired centrally in a dorsal root has on a pre-existent d.r.p. in that root. Such an investigation is complicated by the d.r.p. that this test-volley alone produces. We have confirmed the observation of Barron and Matthews that this d.r.p. is at least as great as that produced by a volley in an adjacent dorsal root; and in every other respect these two d.r.p.'s are similar.

In Figure 10 a d.r.p. is set up in the 10th dorsal root by a volley in the 9th dorsal root, and this d.r.p. is subjected to the action of a maximum volley fired orthodromically into the cord along the 10th dorsal root (see position of electrodes in inset diagram). This latter volley will of course be recorded as a diphasic spike potential (only the declining part of second phase is visible in Fig. 10), but the control observation shows that the second phase (recorded as a negativity of the cord electrode relative to the electrode distally placed on the root) has probably terminated before the initiation of the d.r.p. (cf. 9). The slight dip below the base line in Figure 10 is probably

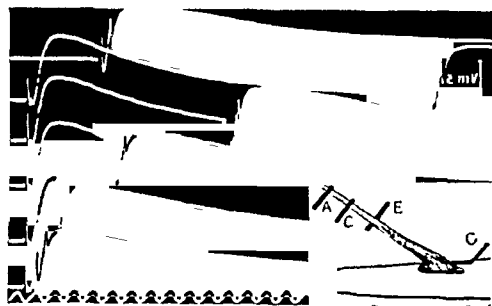


FIG. 10. Upper record is a control d.r.p. set up in D10 by a maximum volley in D10. The diphasic nerve action potential of this volley was too faint for reproduction. In remaining series this D10 volley (orthodromic volley) is fired at various intervals into d.r.p. set up in D10 by a large D9 volley. Note dorsal root reflexes set up by D10 volley. Temp.: 23.5°C. Time: 1 d.v. = 10 msec. Inset shows disposition of electrodes on D10, E earth, G grid, C cathode, A anode.

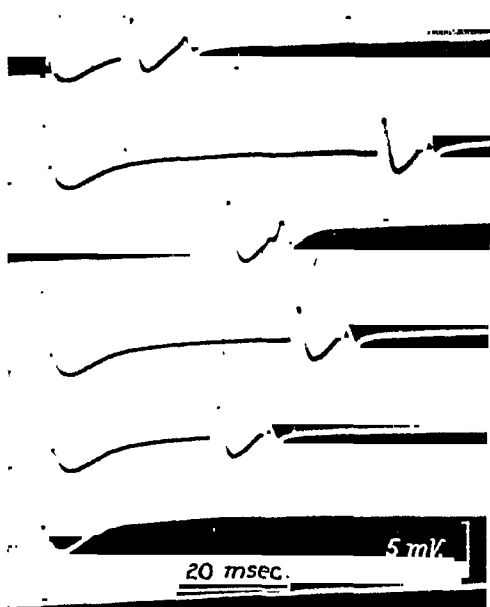


FIG. 12. Series as in Fig. 10 in another experiment with faster recording, initial diphasic spike of orthodromic volley now being shown. D.r.p. initially set up in D10 by a weak D10 volley—control shown in lowest record just above base line. Maximum D10 volley (the orthodromic volley) at various intervals, control being shown in 3rd record (note dorsal root reflexes). Temp.: 14.5°C.

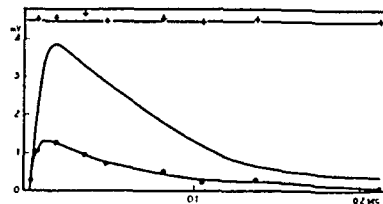


FIG. 11. Series partly shown in Fig. 10. The levels of the d.r.p. undestroyed by the orthodromic volley are plotted (solid circles) as ordinates against the respective intervals after the D9 stimulus as abscissae. The continuous line shows the control d.r.p. set up by D9 alone. The crosses show the potentials of the corresponding orthodromic spikes, while the horizontal line through them marks the average control height of the orthodromic spikes.

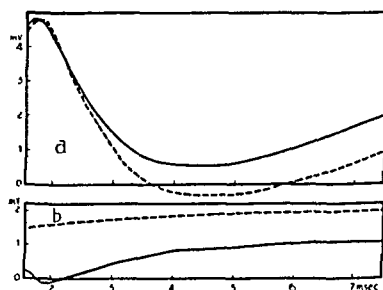


FIG. 13a. Continuous line shows a similar observation to that of the upper-most record of Fig. 12, but taken just before at a higher speed. Broken line shows superimposed control response to orthodromic volley alone. Records only plotted from upward spike to onset of dorsal root reflex. Note slight forward shift of spike relative to control. Base line for both curves is initial base line before stimulation. Time in both is measured from orthodromic stimulus.

b. Continuous line plots difference between two curves of (a), giving time course of reappearance of undestroyed d.r.p. Initial diphasic component is due to forward shift of spike. Broken line shows control course of d.r.p. to initial volley alone, i.e., if orthodromic volley had not been interpolated.

due to an initial inverse recording of the d.r.p. (cf. section A1). It will be observed that at all intervals the orthodromic volley has caused a large destruction of the pre-existent d.r.p., and that the time course of its rebuilding closely resembles the rising phase for the orthodromic volley alone.

Assuming that at all volley intervals the initial dip (see above) is the same size as in the control, the destruction of d.r.p. by the orthodromic volley can be determined, and is plotted in Figure 11 together with the time course of the d.r.p. set up by the 9th dorsal root volley alone. It will be observed that a constant fraction of the d.r.p. is destroyed except during its rapidly rising phase, where the destruction is relatively smaller.

An orthodromic volley has similarly been observed to destroy a fraction of the d.r.p. in all our experiments, but the fraction has varied widely in different experiments and even in different roots in the same experiment, being usually 50 to 90 per cent. These figures are for orthodromic volleys set up by stimuli well above maximum (as tested by the spike potentials). With submaximum orthodromic volleys the destruction is proportionately less. The slowly decaying d.r.p. probably produced by internuncial after-discharge (section F) shows the same destruction as the quickly decaying potential. Likewise a similar fraction of destruction is observed with the slowly decaying d.r.p. of the narcotized cord and also with the d.r.p. set up by a ventral root volley. It is further to be noted that no destruction of d.r.p. has ever been observed when a volley is fired in through a different dorsal root from that in which the d.r.p. is recorded (cf. Fig. 6a; 2, Fig. 5).

A more detailed analysis of the destruction of the d.r.p. and its rebuilding has been attempted in faster records. For example, in Figure 12 the spike of the testing orthodromic volley can be compared with its control record. As would be expected, the initial spike downwards (relative negativity of electrode E in Fig. 10) is unaffected by the pre-existent d.r.p., but the subsequent upward spike (relative negativity of electrode G in Fig. 10) occludes with the pre-existent d.r.p., its height above the original base line being almost the same as in the control observation (cf. crosses in Fig. 11). Usually it is a little lower than the control, and faster records show it to be a little earlier (Fig. 13a). Presumably this is an example of the speeding up of impulses by a background catelectrotonus, and the resulting increased interference between the upward and downward phases would account for the diminution usually observed in the upward spike.

The complete submergence of the pre-existent d.r.p. by the upward phase of the spike might suggest that, as with other catelectrotonic potentials, it suffers complete destruction. Any reappearing d.r.p. would then have to be regarded as being due to new formation. As shown in Figure 12, there is often a brief interval between the orthodromic spike and the d.r.p. it sets up (on the other hand, they may overlap). However, when the orthodromic spike is set up during a pre-existent d.r.p., a reappearance of some d.r.p. occurs during its falling phase, as is typically shown in Figure 13a. Further, this reappearance of d.r.p. seems to overlap with the new formation of d.r.p.

by the orthodromic volley, for the new rising phase is initially a little more steep than in the control record. Subtraction of the control response to the orthodromic volley alone shows approximately the time course of reappearance of the d.r.p. (Fig. 13*b*). It will be seen that in this observation about half of the original d.r.p. has reappeared in this way. A similar time course for reappearance is observed when the orthodromic volley is fired in during the falling phase of a d.r.p.

There would seem to be two possible explanations of this phenomenon of d.r.p. reappearance:

(i) The d.r.p. is partly produced at some central locus which is not accessible to the orthodromic volley. This fraction would thus survive unaffected by the orthodromic volley, but would not be recorded during the summit of a spike produced as a maximum volley of impulses in the dorsal root fibres entering the cord, for under such conditions the electrotonic potential would be unable to spread out from its central site of origin. However, during the declining phase of the spike, the dorsal root fibres regain their polarization and consequently their ability to transmit catelectrotonic potentials; hence the reappearance of the undestroyed d.r.p.

(ii) During the phase of active depolarization (cf. Fig. 9, dotted curves) continuous production of the d.r.p. is occurring and this could account for a rebuilding of d.r.p. in the interval between the orthodromic spike and its initiation of new d.r.p. (cf. the analogous observations of Kuffler on the endplate potential, 22, Figs. 4 and 5). However, this explanation would not be applicable during passively decaying phases of the d.r.p., where the first explanation alone can be applied. That this second explanation applies in part during the active phase of depolarization is suggested by the greater immediate recovery of d.r.p. often observed early in this phase (cf. Fig. 11).

### H. Dorsal root reflexes

A large dorsal root volley is often followed by the discharge of impulses from the cord along this dorsal root and also adjacent dorsal roots (3, 4; 26, 27; cf. Figs. 1, 10, 12, 14). It has been suggested (3; 12, pp. 376-9) that this discharge of impulses is probably fired off by that rapid depolarization of the central terminals of the dorsal root fibres which is observed, after electronic spread, as the dorsal root potential. Several findings of the present investigation give support to this explanation.

1. As would be expected for nerve fibres with well developed accommodation, the steepness of rise as well as the height of the d.r.p., i.e., of the central catelectrotonus, is of significance in initiating the discharge of impulses. For example, the discharge is never observed to be initiated during the last third of the rising phase of the d.r.p. This closely parallels the setting up of discharges of motoneurons by those catelectrotonic potentials which have been called synaptic potentials (14).

2. It has been observed that the dorsal root reflex set up by a testing volley is inhibited by a preceding conditioning volley (26). The following observations show that this inhibition is satisfactorily explained by the hypothesis that the dorsal root reflex is initiated by the catelectrotonic d.r.p.

(i) When the recording electrodes are on a different root from that into which the testing volley is fired, the d.r.p. which this volley sets up is considerably occluded by the d.r.p. already produced by the conditioning volley (Fig. 6*a*; cf. 2, Figs. 5 and 6; 4). Hence the rising phase of its d.r.p. is correspondingly less steep and so less effective in setting up a dorsal root reflex. As would be expected on this explanation, the time course of the

"inhibition" corresponds to the time course of the d.r.p. set up by the conditioning volley.

(ii) On the other hand, when the recording electrodes are on the root into which the testing volley is fired, the testing volley removes a considerable part of the pre-existent d.r.p. produced by the conditioning volley (section G); hence there will be a correspondingly increased rising phase for its d.r.p. This satisfactorily accounts for the smaller inhibitory effect observed. As would be expected, there is an inverse correlation between the effect of the testing volley in destroying the pre-existent d.r.p., and the "inhibition" of its dorsal root reflex by this d.r.p. (Fig. 14a). Again, as in (i) the time course of the "inhibition" cor-

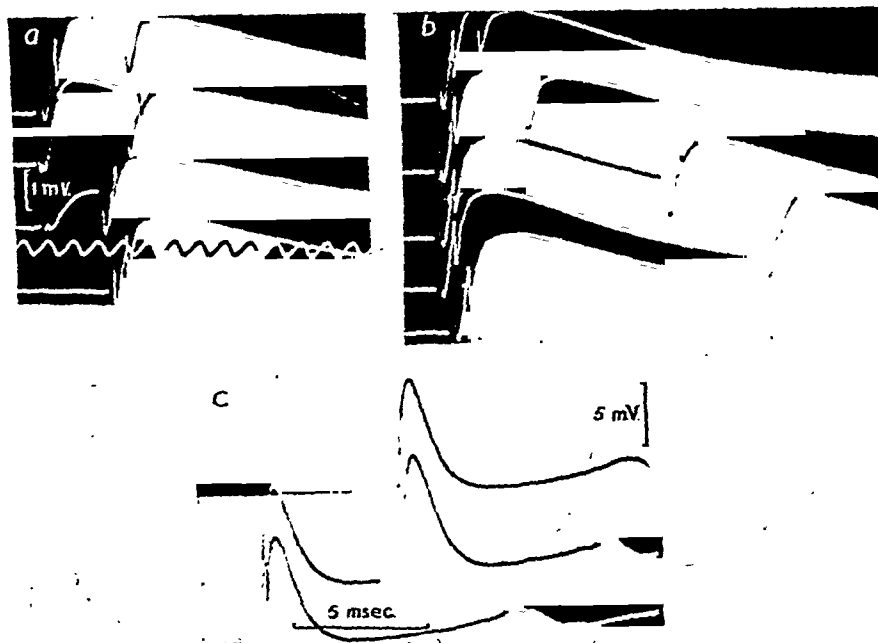


FIG. 14a. Lowest record shows d.r.p. in D10 with dorsal root reflex set up by orthodromic volley alone (spike of orthodromic volley not shown, cf. Fig. 10). Upper three records show that inhibition of this reflex by pre-existent d.r.p. is related to effect of this d.r.p. in preventing new formation of d.r.p. by the orthodromic volley. From above downwards, initial volley was maximum in D10, large in D9 and small in D9. Time: 1 d.v.: 10 msec. Temp.: 23.5°C.

b. Same experiment as above with both maximum volleys in D10 and d.r.p.s and dorsal root reflexes recorded in D10. Lowest record control for 2nd volley alone. Series shows progressively less inhibition of dorsal root reflex as volley interval lengthens. Time and potential scales as for (a).

c. Similar experiment on a different preparation with fast recording. Controls of 1st and 2nd volleys alone shown by lowest and uppermost records respectively.

responds to that of the d.r.p. set up by the conditioning volley (Figs. 10 and 14b). Incidentally, it may be observed that the existence of this lessened degree of "inhibition" of dorsal root reflexes set up by orthodromic volleys affords a striking confirmation of the partial destruction of pre-existent d.r.p. which was inferred from direct observation (section G). If destruction were complete, then no inhibition would be expected.

(iii) Another type of "inhibition" of the dorsal root volley is shown in Figure 14c. If the testing volley is fired in before the conditioning volley has set up its dorsal root reflex, this reflex is delayed until approximately the time at which the testing volley alone sets up its reflex discharge. Presumably this occurs because the testing volley largely destroys



the conditioning volley's d.r.p. before it has initiated the dorsal root reflex. The d.r.p. newly formed by the testing volley then initiates the dorsal root reflex, but it is aided by that part of the conditioning d.r.p. which escapes destruction (cf. Fig. 13b); hence the slightly earlier time of the delayed reflex in Figure 14c than in the control observation for the testing volley alone.

3. The dorsal root reflex is abolished during the deep narcosis produced by 1 in 5,000 nembutal. This effect may be entirely attributable to the concurrent diminution of the d.r.p. (section D), but it is possible that the narcotic also exerts a stabilizing influence on the membrane of the terminals of the dorsal root fibres so that a larger catelectrotonus is needed to initiate impulses (cf. the narcotic action on motoneurone discharge, 14).

4. The much slower rate of rise and lower summit of the d.r.p.'s set up by ventral root volleys (cf. Fig. 2 with Fig. 1) is sufficient explanation of their failure to initiate the discharge of impulses in dorsal root fibres.

In conclusion it may be stated that all the experimental evidence at present available conforms with the hypothesis that the dorsal root reflex is initiated by the same process that gives rise to the dorsal root potential, namely, a catelectrotonus of the central terminals of the dorsal root fibers. There is thus no need to postulate special reflex pathways for the dorsal root reflex (cf. 26). Its "central reflex time" of some 7 to 10 msec. (frog) is satisfactorily accounted for by the time of the rising phase of the d.r.p. These findings should serve as a warning against attempting to calculate the number of synapses in a reflex pathway merely from the value of the central reflex time. It is to be noted that, though the present investigation has been almost wholly on frogs, cats show similar dorsal root potentials (2, 4), so presumably a similar relationship obtains between dorsal root potentials and dorsal root reflexes. Finally, attention may be drawn to the spread of these reflex impulses in the spinal cord. Presumably they traverse all branches of their respective fibres which are accessible to an orthodromic impulse, and so exert on the pre-existent d.r.p. a destructive effect similar to that of an orthodromic volley (section G), an effect which is observed on close inspection of Figures 10, 12 and 14.

### *I. Evidence relating to mode of production of dorsal root potential*

Two alternative explanations have been offered for the production of the dorsal root potential: that it is primarily produced in the terminals of the dorsal root fibres, arising as a special kind of negative after-potential following the propagated impulse (2, 18, 9); that the primary potential is in neurones with which the dorsal root fibres enter into close contact (synaptic or otherwise), and the dorsal root fibres are in turn depolarized by the currents generated by this potential (5, 6, 10, 12).

The following evidence has been cited as supporting the first explanation as opposed to the second. (a) The latent period of the d.r.p. is not shortened by increase in the size of the dorsal root volley. It thus differs from the synaptic transmission of impulses, where such a shortening is observed. (b)

When two dorsal root volleys are fired simultaneously the summed d.r.p. is less than the sum of the d.r.p.'s individually produced. On the contrary, with synaptic transmission of impulses an effect greater than summation is frequently observed (spatial facilitation). (c) Similarly, with two volleys at various intervals, either in the same or in different roots, there is always occlusion of the d.r.p.'s, never the facilitation often observed with synaptic transmission; nor is shortening of the latency of the second d.r.p. ever observed under these conditions.

However, these arguments concern only the synaptic transmission of impulses and take no cognisance of the synaptic potential which the dorsal root volley sets up in nerve cells by trans-synaptic action (14). The latent period of the synaptic potential is unaffected by the size of the dorsal root volley, and two volleys simultaneously or successively never set up a synaptic potential greater or earlier than the summed individual potentials. Usually there is a considerable occlusion, particularly when internuncial after-discharge is intense. Thus the evidence cited above, far from excluding the second explanation (d.r.p. arising secondary to a trans-synaptic potential), actually would be predictable from it.

Further evidence supports the second explanation as opposed to the first.

(d) The synaptic potential arising trans-synaptically has a shorter latent period and a much briefer time course than the dorsal root potential (compare Fig. 7b with 7a). As shown in Figure 9, there is a close resemblance between the rising phases of the synaptic potential set up by a synchronized synaptic bombardment (broken lines) and the calculated curve (dotted lines) for the depolarizing agent setting up the d.r.p. when internuncial after-discharge has been abolished by nembutal. The possible relationship of the synaptic potential to the active depolarizing agent setting up the d.r.p. will be discussed in section J. Comparison of the respective time courses (Figs. 7a and b, 9) shows that the view of Barron and Matthews (2) that the depolarization of the terminals of the dorsal root fibres (as signalled by the d.r.p.) causes the depolarization of the motoneurons (as signalled by the synaptic potential) is no longer tenable.

(e) A dorsal root volley may set up as large a d.r.p. in an adjacent root as in its own root. Barron and Matthews (2) offer an ingenious explanation in terms of ion flow from the active terminals to passive terminals. As already suggested (12, p. 366), the wide distribution of the d.r.p. along the spinal roots would be predicted on the basis of the known spread of the dorsal root volley either along the intraspinal course of the dorsal root fibres themselves, or after internuncial relay. According to the above second explanation, the neurones thus activated would secondarily depolarize all dorsal root nerve fibres in close proximity to them, so giving the observed d.r.p.'s. This effect would be independent of the previous activity or passivity of these dorsal root-fibres, hence the large d.r.p.'s in adjacent roots are simply explained by the overlap of the neurone fields to which their respective fibres are distributed.

(f) The increased and prolonged d.r.p. in the strychninized cord has been shown by Dun (10) and Dun and Feng (11) to be due to a great increase in the internuncial after-discharge (which would act as in (e) above), for it is still present after section of the dorsal columns between the stimulated and the recorded dorsal roots, which interrupts all direct fibre connection.

(g) Veratrine greatly increases and prolongs the negative after-potential of nerve, but it appears to affect the synaptic potential and the d.r.p. only by its action in increasing internuncial after-discharge. In deep narcosis this complication is absent, and then veratrine in concentrations up to 1 in 70,000 has no appreciable action. These potentials therefore are not closely related to the negative after-potential but conform instead to the type of catelectrotonic potentials (cf. the absence of veratrine action on the endplate potential, 16, p. 227; 23).

(h) The observed destruction of the d.r.p. by an orthodromic volley would be predicted if it were a catelectrotonic potential, not if it were a negative after-potential, for in the latter case the full height of the spike would stand above the pre-existent negative after-potential (19), and on decline of the spike no destruction would be observed.

There are, however, some observations which raise difficulties for either explanation of the d.r.p.

(i) The d.r.p. produced by a ventral root volley gives a relatively delayed and prolonged time course for the action of the depolarizing agent, as may be seen by inspection of Figure 6*d*. Simultaneous records from the ventral root show that this volley sets up in the motoneurones a positive potential (17, 20), having a similar rising phase but a decay considerably slower than that of the depolarizing agent (cf. the comparison in Fig. 9). The current flow produced by such a positive potential could only act on the dorsal root terminals as a depolarizing agent (*i.e.*, similarly to the action postulated in (d) for the negative synaptic potential), if it were located in the soma and dendrites of the motoneurones in some diametrically opposite way. This appears improbable, for synaptic endings seem to be dispersed in a random manner over the soma and dendrites of a motoneurone except for the fine dendritic terminals. An alternative explanation could invoke the action on the dorsal root terminals of impulses in the axon collaterals, but it is an *ad hoc* hypothesis without experimental support.

(ii) The partial destruction of d.r.p. by a maximum orthodromic volley (section G) can only be explained by assuming that some impulses of the volley fail to propagate along some of the terminal branches of their respective dorsal root fibres; and that, nevertheless, potentials generated in those terminals would be able to spread electrotonically along the dorsal roots. Such a block is likely to be produced when a large increase in area of a fibre's surface membrane occurs because of profuse branching. This rapid expansion of the surface membrane could cause the safety factor for propagation normally to be below zero. It is to be noted that a similar areal expansion probably explains the block of antidromic impulses at the axon hillock of motoneurones (24, 25).

### *J. Hypothesis concerning origin of dorsal root potentials*

The foregoing evidence strongly suggests that this hypothesis should attempt to explain the generation of the d.r.p. by the synaptic potential. The necessity for this is particularly evident when it is recalled that the d.r.p. is set up when the narcosis is deep enough to block completely the initiation of impulses in the spinal cord (cf. Fig. 7*a* and *b*), leaving only the synaptic potentials of cells directly excited by the dorsal root volley. It is possible, too, that a similar condition obtains for the d.r.p.'s set up by a single impulse in a dorsal root fibre (18).

Recently an hypothesis has been developed which shows how the catelectrotonic junctional potential (synaptic potential or endplate potential) could be set up by the electrical currents which are generated by an impulse propagating in the pre-synaptic nerve fibre up to its termination at the junctional

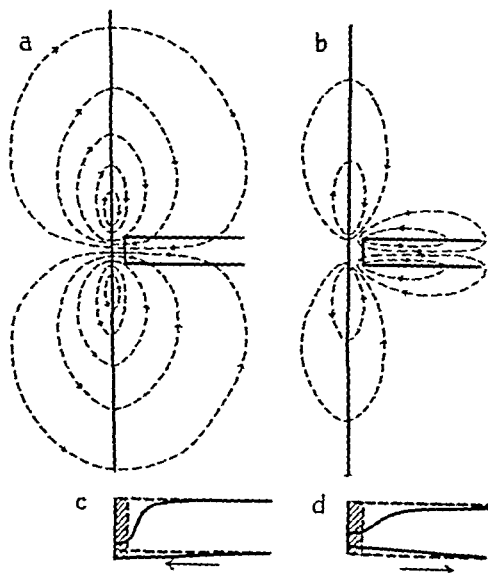
region (15). This hypothesis will now be extended in order to explain how the synaptic potential of nerve cells (internuncial neurones as well as moto-neurones) could set up catelectrotonic potentials in the adjacent synaptic terminals of nerve fibres. The original paper may be referred to for a full statement of the assumptions from which the hypothesis was developed, but it may be mentioned that in general it has been assumed that the membranes have the electrical properties directly determined for axon membranes (cf. 7). In addition, it was necessary to assume that the synaptic region of the effector cell responded by graduated and intense local responses much as does

FIG. 15. Diagram showing in section the termination of a presynaptic fibre (assumed to be a cylinder with a closed end) at a synapse on the surface of a neurone (assumed to be an indefinitely extended plane surface at right angles to plane of paper, and shown in section as a vertical line).

a. Shows the lines of current flow during the rising phase of a synaptic potential initiated by a local response of that part of the surface membrane of the neurone which lies under the nerve terminal. Note descending core current passively produced in presynaptic fibre.

b. Shows lines of current flow during decay of active process setting up synaptic potential. As this current declines, the reversed current is actively set up in the pre-synaptic terminal by the local response at its end.

c, d. The continuous lines show diagrammatically the potentials for the inside and outside of the surface membrane of the presynaptic fibre in conditions a and b respectively. Ordinates are potentials (increasing positivity upwards) of the outside and inside of the fibre membrane, and abscissae the corresponding points of the fibre projected downwards from a and b respectively. The end of the fibre is shown by the hatched section at left. Resting potential levels of the outside and inside of the fibre membrane are shown by the horizontal broken lines. Direction of core current shown by arrows.



a narcotized, refractory or deteriorated axon membrane. In the present hypothesis this latter assumption is extended to the terminal region of the pre-synaptic fibre.

As shown in schematic form in Figure 15a, the catelectrotonic currents flowing during the active phase of the synaptic potential will partly penetrate and flow down the pre-synaptic nerve fibre, giving a diffuse anodal action along its length and a localised cathode at its synaptic terminal. The lowering of resistance at this terminal cathodal focus (7, 21) will serve to canalize the current through this region, and, according to the hypothesis, the consequently intensified cathodal depolarization will set up a large local response at this synaptic region of the nerve fibre (cf. Fig. 15c). Meanwhile, after the

inflection on the rising phase of the synaptic potential (usually about 1 to 2 msec. after its initiation), the currents shown in Figure 15*a* will decrease, and the polarization which they set up in the pre-synaptic nerve fibre, together with the postulated "local response" generated at the terminal, will cause the "core current" of this fibre to reverse with the resulting current distribution of Figure 15*b*. A spreading catelectrotonus, *i.e.*, the dorsal root potential, will be set up by this reversed current flow in the pre-synaptic nerve fibre (*cf.* Fig. 15*d*). This current will increase during the decline of the currents generating the synaptic potential (*i.e.*, during the decline of the actively depolarizing agent calculated for this potential, *cf.* 14), itself in turn declining as the local response at the nerve terminal subsides. In each dorsal root fibre the spreading catelectrotonus will be produced by the summed effect of its many active terminal foci; hence is explained the large size of the d.r.p. According to the hypothesis, it is virtually the time-intensity relationship for the flow of this current which is shown in the dotted curves of Figure 9 as the time course of the actively depolarizing agent. It is to be noted that in Figure 9 the dotted curve rises about 1 msec. later than the synaptic potential (broken line), a relationship conforming with the above explanation and observed in each of the five relevant experiments. It is further to be noted that in this Figure the decaying phase of the synaptic potential bears no relationship to the d.r.p. (either dotted or continuous curve). This phase is merely the recharging of the condensers of the neuron's surface membrane by intrinsic action, and would give little extrinsic current flow if all parts of the membrane recharged at about the same rate; hence the negligible action on the pre-synaptic fibre would be expected.

The decaying phase of the d.r.p. is similarly assumed to be due to the intrinsic recharging of the condensers of the nerve terminal's surface membrane through the membrane resistance. In nembutal narcosis the great slowing of the decay (about ten-fold) is presumably attributable to an increase in the electric time constant of the membrane (*cf.* section F3). This effect is probably brought about by an increase in its resistance, rather than capacity; for membrane capacity has been found to be extraordinarily stable, whereas its resistance shows wide variations under experimental conditions (7). Moreover, narcotics usually decrease membrane permeability to ions (*cf.* 8). It need not be assumed that such an action of nembutal is exerted along the whole length of the dorsal root fibres. If it were restricted, for example, to the *non-medulated terminals in the spinal cord*, there would be such a large surface capacity slowly recharging by intrinsic action that the resulting slowly decaying catelectrotonus would continue to spread electrotonically and so be recorded from the dorsal roots at their origin. It should be possible to test this explanation by direct measurements of the electric time constants of dorsal root fibres and of their central terminals before and during narcosis. It is interesting that no such lengthening of time constant by nembutal is observed for motoneurons and for ventral root fibres (14).

Thus the hypothesis would explain the action of narcotics on the d.r.p.

set up by dorsal root volleys as being largely due to two effects: (i) the diminution or removal of internuncial after-discharge (cf. section Fii); (ii) the lengthening of the time constant of the membrane surrounding the central terminals of the dorsal root fibres. In addition, the diminution which deep narcosis produces in the initial rate of rise of the d.r.p. may be assumed to be secondary to the diminution which the narcotic causes in the synaptic potential directly set up by a dorsal root volley (cf. 14).

The present hypothesis also provides an explanation of the following:

(a) In Figure 15 it was assumed that the synaptic potential was initiated immediately under the terminal of the pre-synaptic fibre, *i.e.*, that the d.r.p. was being observed in the root in which the volley was set up. However, a somewhat similar current flow would occur if the synaptic potential were being set up by activity at a synapse closely adjacent to the pre-synaptic fibre under observation; hence is explained the d.r.p. which is observed in a root adjacent to that stimulated.

(b) The delayed summit of the d.r.p. set up by small dorsal root volleys moves progressively earlier as narcosis is deepened until it reaches the uniform time observed for all volley sizes (section D2). This suggests that the prolonged rising phase is due to the cumulative action of the synaptic potentials produced by internuncial after-discharge, an explanation which is supported by the observations: (i) that the synaptic potentials set up by such small volleys also rise slowly and are prolonged, thus giving evidence of considerable internuncial after-discharge; (ii) that still smaller volleys are regularly observed to set up d.r.p.'s with much earlier summits; (iii) that weak strychnine (1 in 200,000, for example) greatly prolongs the rising phases of the d.r.p.'s.

(c) The flattened summit of the d.r.p.'s set up by large volleys suggests that a ceiling is reached in the depolarization of the central terminals. The hypothesis would suggest that the local responses at the terminals had reached a maximum size. A similar explanation may be offered for the occlusion of the d.r.p.'s set up by two volleys and the small summation observed with repetitive stimulation. Apart from the delayed decline of the d.r.p., which is attributable to internuncial after-discharge (section F), the d.r.p. set up by repetitive stimulation shows no prolonged after-response such as would be expected if the accumulation of some substance, *e.g.*, K ions, played a significant role in its production. However, such a cumulative action would not be expected on the present hypothesis.

In conclusion it may be stated that the present hypothesis has the merit of attempting to unify several diverse phenomena: the potentials recorded in the ventral and dorsal roots under the diverse experimental conditions provided by variations in size of dorsal root volleys, by repetitive volleys and by the actions of nembutal, convulsant drugs, and orthodromic volleys. In particular it is successful in relating the only two prolonged electrical responses that have so far been observed in the deeply narcotized spinal cord, namely, the synaptic potential and the dorsal root potential. It is closely

linked with the electrical hypothesis of synaptic transmission (15); for, if electrical interaction across the synapse is adequate to explain the production of d.r.p.'s, then it is likely to be adequate to explain the production of synaptic potentials, and hence synaptic transmission; and vice versa. In testing these hypotheses the determination of electric time constants of the central nerve terminals and of the motoneurons will be particularly significant. Hitherto no direct determination has been attempted, their evaluation being entirely dependent on the assumption that in their latter parts d.r.p.'s and synaptic potentials are decaying passively. Further investigation of the d.r.p.'s set up by antidromic volleys in ventral roots also is indicated, for hitherto it has not been possible to give a satisfactory explanation of these potentials in terms of the hypothesis.

### SUMMARY

The dorsal root potentials which are set up in the frog's spinal cord either by dorsal or by ventral root volleys have been systematically studied. The results of previous investigations have been confirmed, and in addition the experiments indicate that:—

1. The d.r.p. is a catelectrotonic potential propagated electrotonically from a central focus, and is analysable into an initial active phase and a later phase of passive decay.

2. The d.r.p.'s set up by strong and/or repetitive stimulation of dorsal roots have in addition a prolonged phase due to internuncial after-discharge, which is increased by the convulsant drugs, strychnine, curarine and veratrine, and diminished by the narcotic, nembutal.

3. On the other hand, internuncial after-discharge is not effective in prolonging the d.r.p.'s set up by ventral root volleys, which always show a late phase of passive decay comparable with that of 1, above.

4. Nembutal greatly prolongs (up to 10 times) the time constant of decay of the d.r.p.'s set up by dorsal and ventral root volleys, but has relatively little effect on the rising phases.

5. The d.r.p. recorded in a dorsal root is abolished during the spike of a maximum volley fired in through that root and in part recovers during the decline of the spike, leaving usually 50 to 90 per cent permanently destroyed.

6. The discharges of impulses out along the dorsal root fibres (dorsal root reflex), which often is associated with the d.r.p., has been shown to conform in all respects to the hypothesis that they are fired off by the cathodal polarization of the central terminals of these fibres.

An hypothesis is developed which shows how the synaptic potential set up by the trans-synaptic action of the dorsal root volley could secondarily produce the cathodal focus at the terminals of dorsal root fibres, and hence the spreading catelectrotonus of d.r.p. The mechanism of such a reversed electrical transmission across the synapse is closely related to the mechanism recently postulated for synaptic transmission. This hypothesis explains all the experimental results on the d.r.p. set up by dorsal root volleys, being

particularly satisfactory in regard to the relative time courses of the events. It has not yet been possible to develop it for the d.r.p. set up by ventral root volleys.

The authors wish to thank the Medical Research Council of New Zealand for provision of the technical assistance for this research.

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# INFLUENCE OF HARMONIC CONTENT ON WAVE FORMS OF THE HUMAN ELECTROENCEPHALOGRAM

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IN THE HUMAN EEG one observes asymmetric waves whose forms strongly suggest quasi-in-phase, low order harmonic addition to the fundamental frequency. Isolated wave forms having such characteristics are recognized in nearly all EEG recordings. In a certain group of subjects who show clinical "psychomotor" seizures (2) random, variable length sequences of approximate square wave discharges are recognized. It is the purpose of this presentation to show that these asymmetric waves in general, and square waves in particular, are more likely to arise from the compounding of fundamental and harmonic oscillations than from direct generation in certain groups of cortical cells.

## METHOD

All EEGs were recorded with an A. M. Grass 6 channel electroencephalograph. Bipolar and monopolar derivations were used in all recordings. The electrodes employed

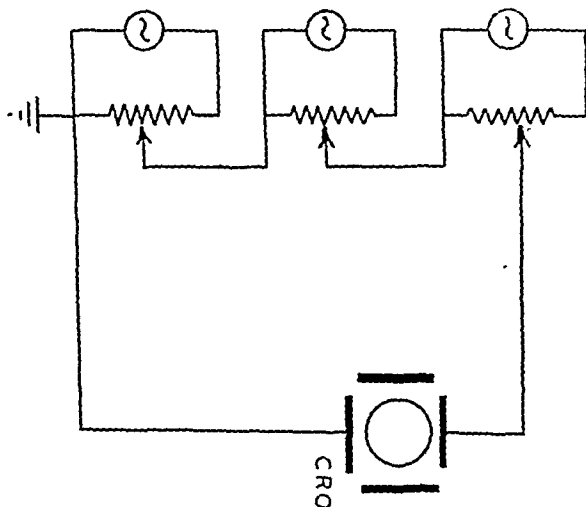


FIG. 1

in this work consisted of small, flattened solder pellets. Certain of the physically (artificial) compounded waves were recorded with the Grass inkwriters through the power stage. Other physically compounded waves were recorded on film from a DuMont type 208, cathode ray oscillograph. The electric oscillations were generated by three Hewlett-Packard audio-frequency oscillators (types 200AD, 200D and 200B). The oscillators were coupled in series as shown in Fig. 1 (3,4).

When a change in physical conditions of a system gives rise to phenomena that may

be depicted as waves, the number of variations in a unit time is designated as the *frequency* of change. The basic rhythm is called the *fundamental* frequency. Simple integer multiples of the fundamental frequency are called *harmonics*. Hence, if the fundamental frequency (or first harmonic) is 10 waves per second, the second harmonic is  $2 \times 10 = 20$  waves per second, the third harmonic is  $3 \times 10 = 30$  waves per second, etc.

The gross effect of the physical addition of low order even (second and fourth) harmonics to the fundamental frequency is to emphasize the amplitude and sharpness of the downstroke of the fundamental wave and to develop an oscillatory plateau on the opposite



A

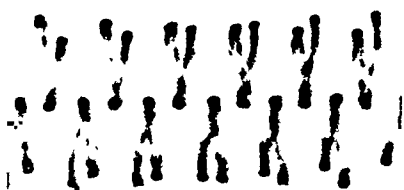
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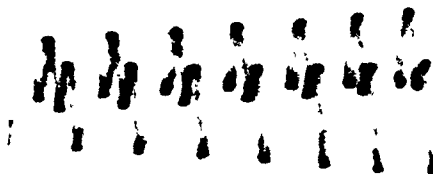
B



C



D → 1/6 SEC ←



E



F

FIG. 2

peak portion of the original wave. Roughly this asymmetric contour approaches the form of a square wave. The result of physically compounding 10 and 20 per second waves is seen in Fig. 2a. Figure 2b is the resultant of physically adding 5 and 10 per second oscillations. When to the fundamental the second and third harmonics are added, the asymmetry and square wave characteristics are more pronounced, Fig. 2c. If, however, only the third harmonic is added to the fundamental a symmetrical wave form results, Fig. 2d. These descriptions hold precisely when the compounded waves are in phase, that is, when each component wave starts its upstroke simultaneously from a neutral position. If the physically compounded waves are out of phase the resultant wave form differs from the above. The extent of difference depends on the degree of out of phaseness, Fig. 2e. From the figure it is seen that the plateaus change their positions relative to the main

peaks and troughs. This is the direct expression of phase change of physically compounded waves. In order for these harmonic effects to take place, the amplitudes of all component waves must be of a similar order of magnitude—within an arbitrary ratio of one to three quarters that of the fundamental. When non-harmonic waves are added to a sinusoidal oscillation, one observes a superimposition phenomenon in which the smooth contour of the fundamental frequency is destroyed. The degree of maintenance of the original contour is a function of the relative frequencies and amplitudes of added waves. The addition of a 10 and 27 per second train is seen in the oscillogram of Fig. 2f.

The usual "normal" human EEG is completely dominated by 10 per second waves of sinusoidal form. Certain individuals, however, show much rhythmic 20 per second

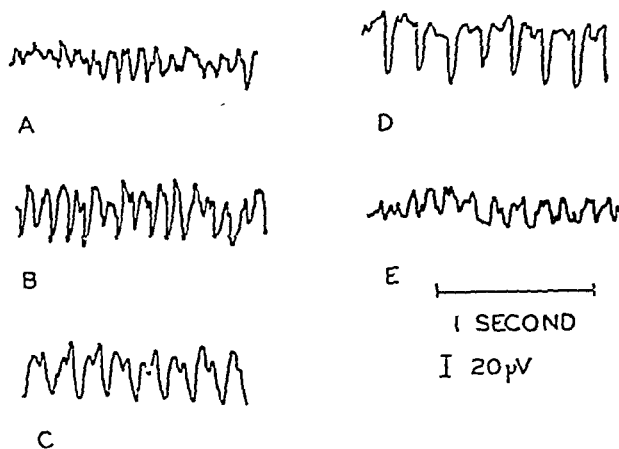


FIG. 3.

activity along with the fundamental 10 per second frequency. In most of these latter cases the amplitude of the fundamental frequency is 2 to 3 times that of the second harmonic. Patterns similar to that of Fig. 3a and b are often observed under these conditions. In this figure the phase relationship of the compounding waves is not well maintained. This is manifest by the inconstant and shifting plateaus.

Under conditions of abnormal brain physiology, either focal or general, a low frequency potential output is characteristic of the EEG. When 5-6 per second waves occur in volleys they are occasionally observed to compound "spontaneously" with their second harmonic to give rise to well defined square waves similar to those of Fig. 3c and d. Figure 3c shows the result of a spontaneous combination of a 5.5 and 11 per second oscillation in a human EEG. Phase shift is obvious. Figure 3d delineates a spontaneous addition of a 5 and 10 per second wave train. In this sample, along with the phase shift a superimposed anharmonic frequency is observed. Figure 3e is the resultant of the spontaneous addition of 7, 14 and 21/sec. waves in a human EEG. Between the intervals of square wave output this EEG showed more rhythmic, and higher voltage 21 per second activity as compared with the 14. It will be seen from the figure that the troughs show minimal plateau formation and that the wave forms in general show some square wave symmetry. This is in keeping with the phenomenon of addition of odd (third) harmonics as noted above.

## DISCUSSION AND CONCLUSIONS

It is clearly demonstrated that the compounded harmonic waves generated by physical (electric) oscillators and the compounded harmonic

oscillations of bioelectric systems, as observed "spontaneously" in certain human EEGs, have similar contours. This points to a similar electrical mechanism operating in each system. Furthermore, this strongly suggests that asymmetrically peaked waves in general, including square waves, are not intracellular phenomena, hence are not uniquely developed potentials—that is, unless a single neurone is found capable of generating two or more autonomous, and simultaneous, frequencies of oscillation. To date such phenomena have not been observed. It is apparent that the smallest known unit capable of giving rise to the observed electric activity must be two discharging nerve cells. However, in the light of ephaptic phenomena and pacemaker mechanisms in general (1) it is most probable that the asymmetric wave forms are the resultant combination of the electric output of two functionally independent relatively large aggregates of cells.

That clinically there is a fairly well defined group of subjects who give rise to bursts of square wave activity is worthy of much consideration. Further study will be necessary to determine whether the individuals subject to clinical "psychomotor" seizures have an exceptionally well developed intercellular synchronizing (phasing) mechanism that is essential for the production of square wave discharges. However, the prominent *phase shifting* and the random nature of the square wave discharges, even in clinically appropriate subjects, points to a statistical chance phenomenon in an individual who generates a fundamental frequency with relatively high voltage harmonics.

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# AN INHIBITORY MECHANISM IN THE BULBAR RETICULAR FORMATION

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ACCORDING to the doctrine that the nervous system is organized as a series of levels, that portion of the brain stem lying immediately above the spinal cord, and described as bulbar, has traditionally been regarded as a level contributing excitatory influences to motor outflows. The basis for this view is the observation, first made by Sherrington (11), that decerebration leaving only the bulbar part of the brain connected with the cord, is followed by a state of over-activity or rigidity of the anti-gravity muscles.

It has not been widely recognized that this bulbar part of the brain stem, in addition, contains a mechanism capable of exerting a general inhibitory influence on motor activity. In the experiments to be reported, this inhibitory influence has been demonstrated by observing the effect of bulbar stimulation upon reflexes, upon decerebrate rigidity and upon responses evoked from the motor cortex.<sup>2</sup>

## METHODS

In cats under chloralosane anesthesia, the blink, flexor and patellar reflexes were evoked respectively by a signal magnet, an inductorium and a solenoid, operating recurrently on a timing circuit, and the excursions of the lid and legs were recorded on a kymograph. Other animals, observed visually, were decerebrated under ether by the anemic method, but using a single parapharyngeal approach to ligate the "internal carotid" and basilar arteries. In other cats under chloralosane, the motor cortex or internal capsule were excited and the leg movements were recorded.

In each animal, the lower brain stem was stimulated, the fine "bipolar" electrodes being oriented with the Horsley-Clarke technique. Sixty cycle, sine wave current, at 3 to 5 r.m.s. volts, was employed routinely, the threshold for evoking responses comparing favorably with that determined with a Goodwin stimulator at higher frequencies. Microscopic examination of frozen sections of the explored area in each case permitted identification of the sites stimulated.

## RESULTS

*Bulbar inhibition of reflex activity.* Bulbar stimulation during reflex activity was found to inhibit the reflexes. In the record shown in Fig. 1A, the flexor reflex of the foreleg (a), the patellar reflex of the hindleg (b) and the blink reflex of the eyelids (c) were reduced or abolished by bulbar stimulation during the period marked by the signal (d). These reflexes, initiated respectively by nociceptive, proprioceptive and tactile stimuli, involve muscles—flexor, extensor, and posturally indifferent—distributed over the length of the body. The bulbar inhibitory influence thus appears to be a

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

<sup>2</sup> Preliminary accounts have been published (7 and 8).

general one, not limited in its action to functionally specific or to topographically circumscribed reflex acts.

These reflexes were not invariably inhibited together, however, and Fig. 1B records an instance in which the flexor reflex was not susceptible to

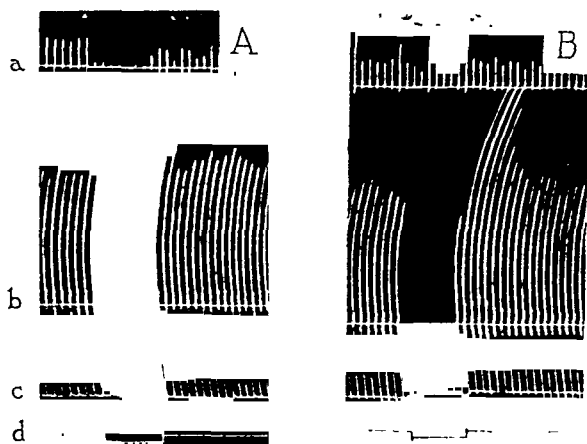


FIG. 1A and B. Kymograph records of the effect of bulbar stimulation (d) on the flexor (a) patellar (b) and blink (c) reflexes evoked at 2 sec. intervals.

bulbar inhibition effective against the patellar and blink reflexes. The bulbar inhibitory effect was frequently followed at the cessation of the stimulus by a subsequent augmentation of whatever activity was proceeding, an example

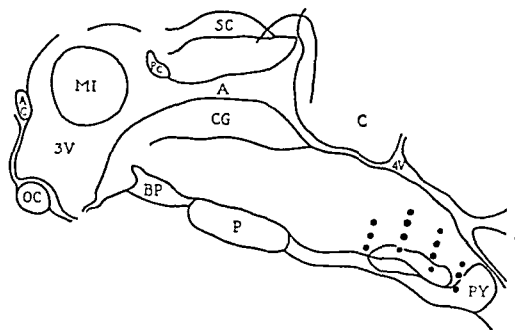


FIG. 2. Midsagittal reconstruction of the cat's brain stem upon which are projected in black circles the bulbar sites whose stimulation inhibited the patellar reflex.

Abbreviations for Figs. 2 and 3 are as follows: A—aqueduct, AC—anterior commissure, BP—basis pedunculi, C—cerebellum, CG—periaqueductal grey, F—facial nucleus and nerve, H—hypoglossal nucleus and nerve, MI—massa intermedia, MLF—median longitudinal fasciculus, OC—optic chiasma, P—pons, PC—posterior commissure, PN—posterior column nuclei, PY—pyramidal tract, R—inferior reticular nucleus, SC—superior colliculus, T—nucleus of the spinal fifth tract, TS—tractus solitarius, V—vestibular nucleus, 3V—third ventricle, 4V—fourth ventricle.

of which is seen in Fig. 1B, b in the case of the patellar reflex, and in Fig. 1A, c in the case of the blink reflex.

In the instances shown in Fig. 1, stimulation of the left side of the medulla was observed against reflexes of the left lid and legs. Whenever it was tested, however, bulbar stimulation was found to inhibit reflexes on both sides of the body.

The general location of the bulbar area inhibiting reflex activity is shown in Fig. 2, projected upon a mid-sagittal reconstruction of the cat's brain stem. Points whose stimulation inhibited the patellar reflex, indicated by black circles, are seen to be distributed in the central portion of the lower brain stem, in what is called the bulbar reticular formation.

The precise locations of the effective points are illustrated in Fig. 3, on

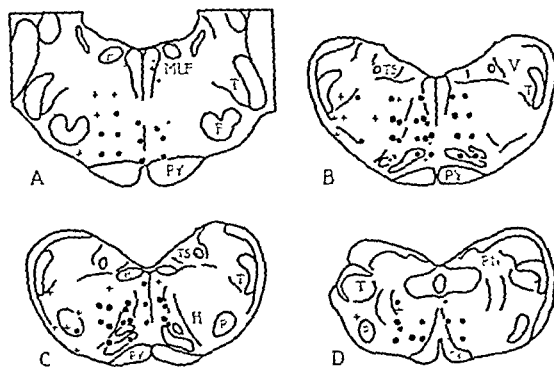


FIG. 3A-D. Four transverse sections through the lower brain stem of the cat, at the levels indicated in Fig. 2, showing the location of bulbar points whose stimulation:—inhibited the patellar reflex (black circles), was without effect upon it (dots), or increased its excursion (plus symbols).

four transverse sections at the levels indicated in Fig. 2. Inhibition of the patellar reflex is seen to have been elicited by exciting a rather long antero-posterior extent of the bulbar reticular formation, chiefly its ventromedial part.

The dots and plus symbols in Fig. 3 indicate points whose stimulation did not inhibit the patellar reflex, and if their distribution is examined, most of the lateral reticular formation and the sensory systems bordering it—the trigeminal, vestibular, vagal and posterior column nuclei—can be ruled out as contributing to the effect. The inferior olive which lies immediately ventral to the excitable area also appears without relation to the inhibitory responses for they have been elicited without impairment after olivectomy (14).

This bulbar inhibition of reflex activity has been obtained after decerebellation and transection through the front end of the medulla and so does not result from the activation of ascending connections to the cerebellum or higher parts of the brain. That it does not result from stimulating connec-



tions from rostral regions simply descending through this area has not been so certainly determined, but no such general inhibitory influence has yet been obtained by the stimulation of more rostral brain stem levels.

From points in the lateral portion of the medulla and others on the periphery of the inhibitory field, increase of the excursion of the patellar reflex resulted from bulbar stimulation (Fig. 3, plus symbols). At least some of these latter responses appear to have resulted from activating facilitatory pathways descending from higher levels (10). If such facilitatory connections in addition pass through the inhibitory field, the frequent appearance of subsequent augmentation after inhibition (Fig. 1) might be attributed to the activation of intermixed inhibitory and facilitatory elements. Such subsequent augmentation might, on the other hand, represent a release from inhibition temporarily depressed after activity.

*Bulbar inhibition of decerebrate rigidity.* It is evident from the above that bulbar stimulation can inhibit phasic reflex activity; it is equally effective in inhibiting tonic reflexes. Decerebrate animals were observed in the supine position with their rigidly extended legs in the air. During bulbar stimulation in the area indicated in Figs. 2 and 3, extensor tone was lost, the legs became flaccid and collapsed, were flail-like to manipulation, and reflexes could not be elicited. At the conclusion of stimulation, extensor hypertonus and other reflex activity promptly returned, the former sometimes with a snap and gain resembling rebound. On stimulating one side of the medulla, the loss of tone and reflexes was complete in all legs except the contralateral fore in which it was but partial.

*Bulbar inhibition of cortical motor response.* One further type of motor activity against which bulbar stimulation has been tested is the response evoked from the motor cortex. In Fig. 4A, flexion of the fore (a) and hind (b) legs, induced by exciting the motor cortex at 2 sec. intervals, was inhibited by bulbar stimulation during the period marked by the signal (c). The site of inhibition here is evidently spinal rather than cortical, for in Fig. 4B flexion of the hindleg (a) induced by activating descending fibers from the motor cortex in the internal capsule (c) was also abolished during the period of bulbar stimulation (b). A similar record, shown in Fig. 4C, illustrates the subsequent augmentation of cortical motor response which may follow its inhibition by bulbar stimulation.

Inhibition of cortical motor response was elicited by stimulating the same bulbar reticular area (Figs. 2 and 3) effective against reflex activity and decerebrate rigidity. In the records shown in Fig. 4, bulbar stimulation inhibited leg responses on the same side of the body, evoked in turn from the opposite motor cortex. In instances in which it was tested, however, inhibition from stimulating one side of the medulla was effective against cortical motor responses on both sides of the body.

*Descending inhibitory pathway in the spinal cord.* Inhibition of reflexes, decerebrate rigidity and cortical motor response was, then, bilateral from stimulation of each side of the medulla. Ipsilateral inhibition could, how-

ever, sometimes be obtained at a lower threshold, or with a given current strength was more complete. This does not necessarily mean that the responsible reticulo-spinal connections are predominantly uncrossed; they might, on the contrary, decussate at the bulbar level, the crossed fibers possibly being more readily excited than the ipsilateral cell groups.

The distribution of points yielding inhibition of the patellar reflex at the lower end of the medulla (Fig. 3D) suggests that efferent inhibitory connections descend in the ventral portion of the spinal cord. This is supported

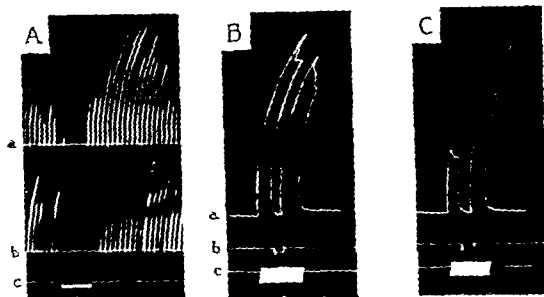


FIG. 4A. Record of the effect of bulbar stimulation (c) upon flexion of the fore (a) and hind (b) legs, evoked from the motor cortex.

FIG. 4B and C. Records of the effect of bulbar stimulation (b) upon flexion of the hindleg (a) evoked from the internal caudate (c).

by instances in which bulbar inhibition of the knee jerk was tested after thoracic cord lesions. Interruption of the posterior column or dorsal part of the lateral column was without effect, but the response was impaired greatly by section of the antero-lateral portion of the cord.

*Coincidental responses.* Depending upon the sites from which they were elicited, the bulbar inhibitory responses were frequently accompanied by respiratory changes (9), vasomotor alterations (13), pupillodilatation (3) and hypoglossal nerve reactions. It was evident in the course of the experiments that these bore a coincidental and not a causal relation to inhibition.

### DISCUSSION

The results described above indicate that the bulbar segment of the brain stem contains neural elements capable of exerting an inhibitory influence on a wide variety of motor performances. While this bulbar function has, to say the least, long remained in obscurity, collateral support for its existence can be found in both old and recent investigations. Deductions drawn from cervical fracture cases led Hughlings Jackson (4) to attribute an inhibitory function to the bulbar region, to which he so aptly referred<sup>2</sup> as "the highest center of the lowest level."

In a study of the early development of behavior in the frog embryo,

<sup>2</sup> And characteristically, in a footnote.

Wang and Lu (12) found initially repetitive spinal motor activity to become suppressed by the maturation of a higher inhibitory mechanism, which by transection experiments was localized in the hindbrain. It is not yet clear, however, that this localization can be applied to the mammalian brain, for Barcroft and Barron (1) described an analogous series of events in the maturation of behavior of the sheep embryo, but located the developing inhibitory mechanism in the forebrain. It has long been known that stimulation of the cerebellum is capable of inhibiting motor activity, and Hare, Magoun and Ranson (2) showed this inhibitory influence to be mediated by cerebello-bulbar connections.

Of the greatest relevance are the observations of Keller (5), that animals maintained into the chronic state after pontile transection of the brain stem exhibit an enduring generalized atonia and absence of some of the more complicated spinal reflexes. It is difficult to explain these symptoms as resulting simply from a loss of excitatory innervation, and, as Keller suggests, they would appear best accounted for by the existence of an active inhibition proceeding from some site below the transection. Since decerebellation did not alter the result, it is not illogical to assume that the bulbar inhibitory mechanism, outlined above, had by appropriate isolation been released and was maintaining the neuraxis below it in a state of reduced activity.

The role which the bulbar inhibitory mechanism plays in the management of motor activity in the intact animal awaits further study. It is likely that it is involved in cerebellar function (2), and its relation to the regulation of motor activity by the cerebral cortex has already been indicated by McCulloch, Graf and Magoun (6), who have reported the reception by the bulbar reticular formation of a descending projection from cortical area 4-S, and suggest that this reticular area constitutes the brain stem relay in an inhibitory extrapyramidal system.

#### SUMMARY

Electrical stimulation of the lower brain stem of the cat has revealed a bulbar area capable of inhibiting motor activity whether initiated reflexly, in decerebrate rigidity or from the motor cortex. The excitable region is distributed in the bulbar reticular formation, chiefly its ventromedial part, and efferent connections descend from it in the ventral part of the cord.

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# MORPHOLOGY AND CONDUCTION OF BIPOLAR DORSAL ROOT GANGLION CELLS OF SELACHIAN FISHES\*

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THE EXTRASPINAL sensory cells of vertebrates consist principally of two types, the bipolar and the T-shaped cells. Multipolar cells are reported as occurring in most groups but are nowhere abundant and their sensory nature is questionable. Much has been written on the relation of the bipolar to the T-shaped cell and it seems clear that the latter is, in the ontogeny of the higher classes, derived from the former (for a review of the extensive literature see Stöhr, 3). Little is known of the functional significance of these two types of cell morphology. The investigations of Erlanger, Bishop, and Gasser (1) indicate that conduction over the T-shaped cells does not involve the cell body nor the single fiber proximal to the dichotomy and that a very slight delay is involved in passage of a volley of impulses through the dorsal root ganglion. While bipolar cells occur in the extraspinal sensory ganglia of the higher vertebrates (albeit infrequently) and in certain situations in the central nervous system, nothing has been determined of the conductile properties of a system consisting of two fibers joined by a cell body. That all vertebrates above the sharks have replaced the primitive bipolar cell would indicate that some deficiency in its function to the organism exists. With this general problem in mind, the following observations were undertaken on the conductile properties of the bipolar dorsal root ganglion cells of the selachian fishes.

## MATERIALS AND METHODS

Through the generosity of the Hopkins Marine Station and with the cooperation of the director, Dr. L. R. Blinks, the cathode-ray oscillograph, condenser-coupled amplifier, and sweep synchronized thyatron stimulator used in my laboratory were set up at Pacific Grove. The species studied were *Raja inornata*, *Raja bimaclulata*, and *Squalus suckleyi*. All observations, figures and diagrams in the following pages refer to *Raja inornata*. Running sea-water was conducted through the gills of the fishes while they were in the laboratory except during the periods when recordings were being made. Curare (Intocostrin, Squibb) was used in place of an anesthetic. The dosage was adjusted to immobilize the fish and was administered intracardially. Observations were made on the first few segmental nerves to the pelvic fins to take advantage of the elongation of dorsal roots which characterizes the caudal portion of the spine. Tissues were removed for examination and stained with Delafield's hematoxylin and eosin, Bodian's silver protargol, and the osmic acid impregnation methods.

## OBSERVATIONS

*Morphology of bipolar cell.* In the species studied, simplicity characterizes the spinal ganglia. All of the cells were found to be bipolar. The size of all the individual cells of a dorsal root ganglion from the body region

\* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

studied has been calculated from measurements made with an ocular micrometer. The material had been formalin fixed and subsequently stained in 1 per cent osmic acid. On the assumption that the cells were ellipsoidal in shape (true of all but the smallest cells) measurements of the long (a) and of the short (b) diameters in micra and employment of the formula

$$V = 4/3\pi a^2b$$

gave the series of cell volumes plotted in Figure 1.

As shown in the osmic acid stained material (Fig. 2) the blackened myelin sheath of the fibers is reflected over the cell body where it forms a thin

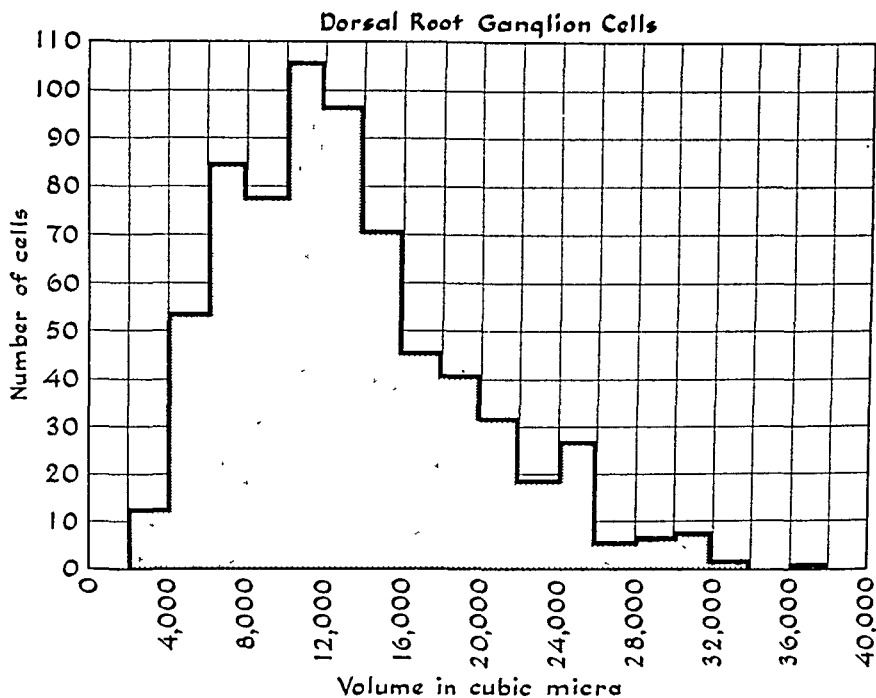


FIG. 1. Frequency distribution curve of volume of dorsal root ganglion cells, based on assumption of ellipsoidal shape.

covering. The irregularities of this layer which are seen in many of the cells may be artifacts; in certain favorably cut cells only a thin even layer of black material is shown. The neurolemma, seen in the osmic acid material as a yellow lucid membrane, is also reflected from the fiber over the cell body. The nuclei of the Schwann cells, clearly seen in the hematoxylin and eosin preparations, continue as an unbroken, though sparse series over the cell body. Very constant is the appearance of an outer membrane which lies external to the neurolemma at the fiber-cell junction. A space is formed between this outer layer and the neurolemma. Because the hematoxylin and

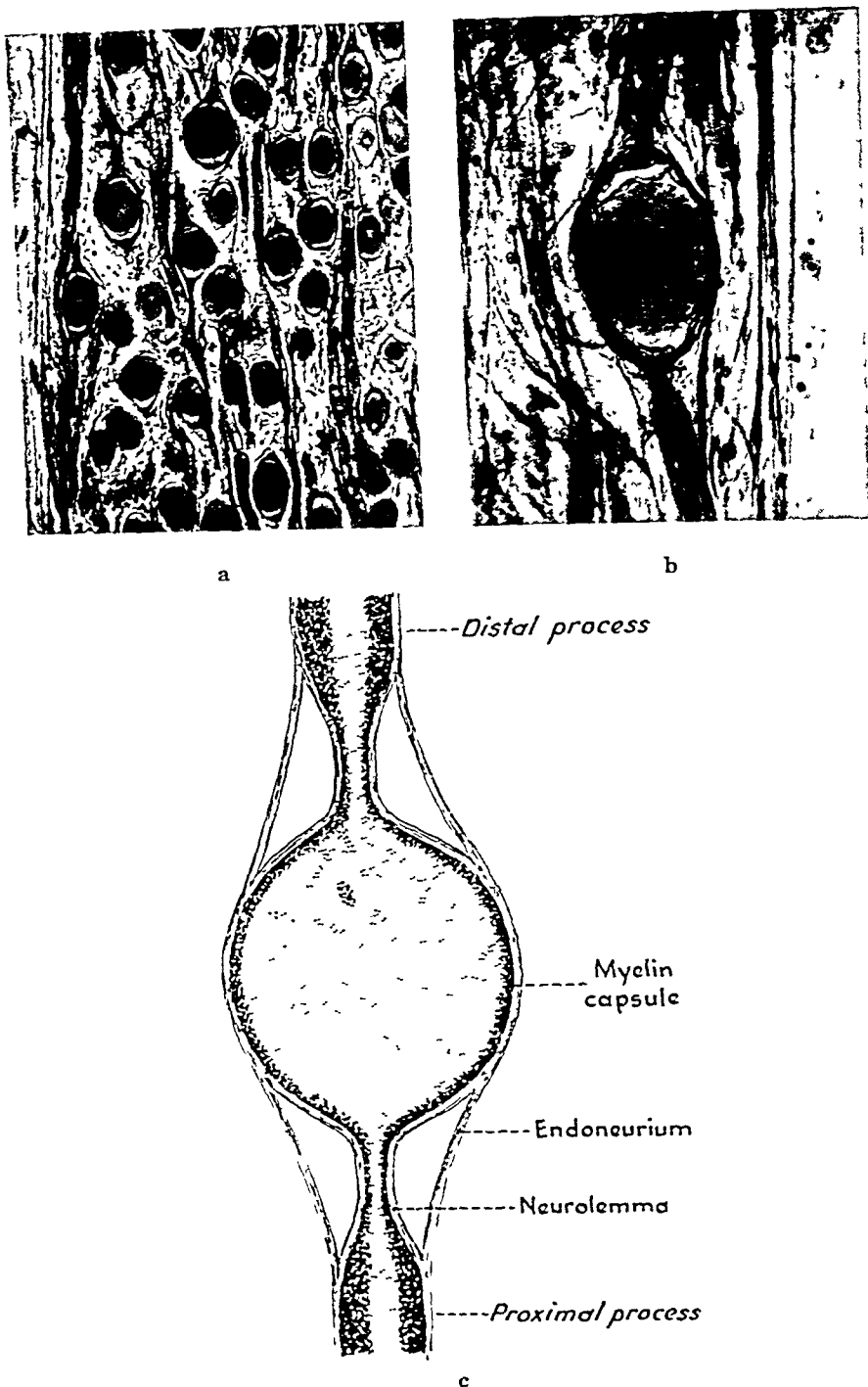


FIG. 2. Osmic acid preparation of dorsal root ganglion showing bipolar cells. *a*, lower powered field (X90). *b*, higher magnification showing details of an advantageously sectioned cell (X300). *c*, idealized sketch of cell showing relations of myelin, neurolemma, and endoneurium.



eosin stained preparation show this in a more diffuse form, it is interpreted as the endoneurial condensation of the connective tissue and not a derivative of the neurolemma. The nerve fiber proper may show a slight constriction at the junction with the cell but no interruption between the cytoplasm of the cell and the axoplasm of the fiber is to be seen. It was not possible to detect any differences anatomically between the proximal and the distal

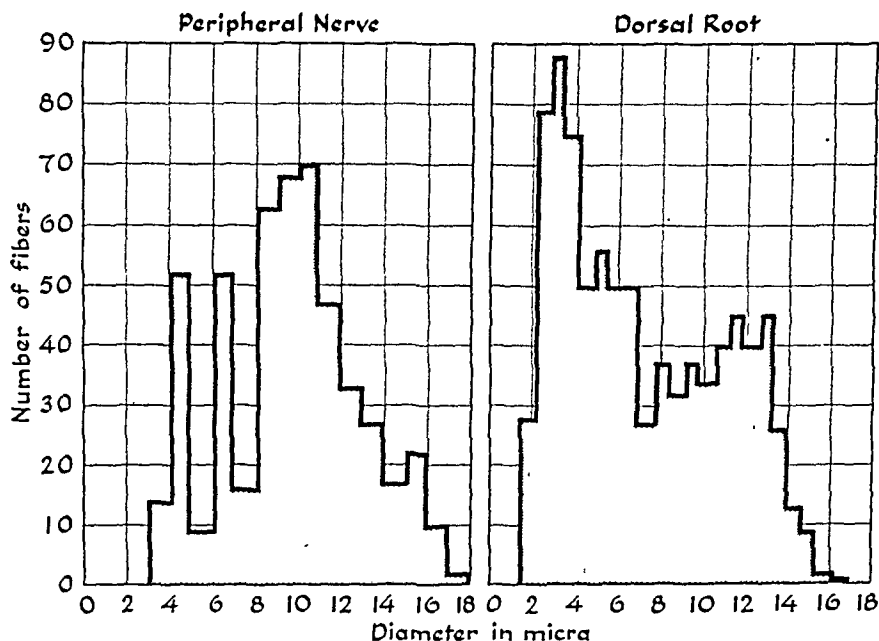


FIG. 3. Frequency distribution of fiber diameters of peripheral nerve, dorsal root.

process of the cells, nor was there a tendency for the nucleus, in the fixed material, to occupy any definite position within the cell.

No deviations from the bipolar shape was noted, though it is possible that among the smaller cells (ca. 2,000 cu.  $\mu$ ) exceptions do occur. Inspection substantiated the fact that the larger cells had processes of larger diameter; no measurement of this relationship was attempted.

*Fiber size spectrum of peripheral nerve and dorsal root.* For an understanding of the multimodal conduction potential (detailed below), size frequency curves were constructed from measurements of the fiber diameter in a peripheral nerve and in a dorsal root. As all material had been formalin fixed, some allowance must be made for shrinkage. The figures are, therefore, minimal. The population represented in Figure 3, being from a total count of a dorsal root, may be expected to contain not only the equivalents of the mammalian A fibers, but of C and possible B fibers as well. On the basis of the investigations of Gasser and Grundfest (2) it is probable that the poten-

tials of Figures 4-6 are mediated by the fibers shown in Figure 3 as the mode extending from  $7\mu$  to  $17\mu$  and probably extending submerged down to the lower diameters of  $2\mu$  or  $3\mu$ .

*Conduction in dorsal root ganglion.* A stimulus, equivalent to that necessary to fire the mammalian A fiber group, applied to a peripheral nerve, initiates a conducted potential which may be recorded at the dorsal root

FIG. 4. Conduction potential of dorsal root following stimulation with shocks of increasing strength applied to peripheral nerve.  $\longrightarrow$

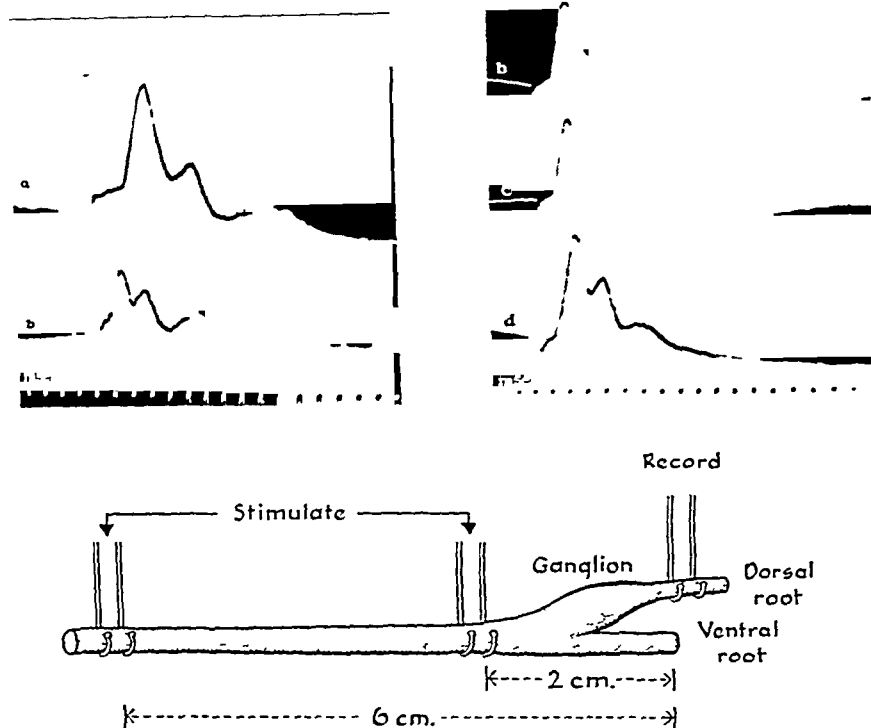


FIG. 5. Conduction potential of dorsal root following stimulation of peripheral nerve. *a*, conduction distance 6 cm. *b*, conduction distance 2 cm.

(Fig. 4). This potential exhibits three clear modes. That these are the expressions of three separable groups of fibers is shown by their behavior following stimuli of varying strength and their altered relations with one another as the conduction distance is changed. The minimal effective stimulus to the nerve results only in the appearance of the first mode. As the stimulus is increased, the second and finally the third are brought into prominence. No attempt was made to investigate the fibers of higher threshold (equivalents of mammalian C fibers).

Alteration in the conduction distance reveals the separate nature of the modes and allows computation of the conduction speed. In Figure 5*a* and *b*, where the conduction distances are 6 cm. and 2 cm. respectively, the latency of the first mode in *b* subtracted from the latency as measured in *a* denotes the period in which the mode has traversed 4 cm. This may be calculated at 36 meters per second. Similarly, the second mode represents fibers with an average speed of conduction of 14 m. p. s.; the third, 8 m. p. s.

The form of the conducted potential in the dorsal root is essentially duplicated in recordings made involving the nerve alone. In Figure 6 may be seen



FIG. 6. Conduction potential in peripheral nerve 3 cm. from point of stimulation.

the same three modes as in Figures 4, 5. Nor does the direction of conduction affect the shape of the potential; experimentation revealed that the dorsal root ganglia conduct equally centripetally or centrifugally.

### DISCUSSION

The meaning of the evolution of the extraspinal sensory cell in the vertebrates is not apparent from its morphology or its conduction properties. There is a tendency for the cells to lose their simple bipolar nature and to form the T-shaped unipolar cells seen in all but the lower fishes. It would seem at first consideration that the change-over might reflect an inability of the bipolar cell to conduct centrifugally or to transmit impulses in a direct manner. It is known that with the exception of a slight delay (Erlanger, Bishop, and Gasser, 1), the T-shaped cell conducts as does a simple fiber. In the foregoing paragraphs, observations have been detailed which indicate that the bipolar cell as seen in the selachian fishes is an efficient conductor and is not directionally oriented. It must be admitted that the equivalence of the two cells is still open to question. The continuation of the myelin covering over the cell body and the formation of a marked endoneurial membrane around the cell may be specializations of the sharks which raise the effectiveness of the cell. These formations may represent independent solutions to the problems posed by the simple bipolar cell in the primitive vertebrates and thus account for the curious survival of this type of ganglion. Also, we may not assume that important physiological differences between the two cell types do not occur for the matter must be better explored than at pres-

ent. Thus, the transmission of repetitive impulses and the sensitivity to oxygen tension might be factors which make one cell type superior to the other. Only continued investigations of these cells will reveal such differences.

### CONCLUSIONS

1. The dorsal root ganglia of *Raja* and *Squalus* among the selachian fishes are made up exclusively of bipolar cells.
2. The large bipolar cells are covered with a myelin layer and in addition to the neurolemma capsule have a substantial endoneurial covering.
3. The volume of these bipolar cells ranges from 2,000 to 38,000 cubic micra.
4. Conduction of single volleys over the dorsal root ganglion is simple and proceeds either centripetally or centrifugally.
5. The dorsal roots and peripheral nerves transmit activity in a multimodal conduction potential. The conduction speeds of the three most rapid groups are 36, 14, and 8 meters per second.
6. Size distribution curves of fiber diameter in peripheral nerve and dorsal root are given.

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# A MIDBRAIN MECHANISM FOR FACIO-VOCAL ACTIVITY

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LITTLE is known concerning neural mechanisms involved in integrating the performance of the cranial musculature in complex acts. Facio-vocal activity is an example of such behavior, exhibited by most higher animals, including man, in the expression of emotion.

Clinical cases in which the two were dissociated by small brain lesions, led Monrad-Krohn (9) and Wilson (11) to suggest that the neural control of the facial and vocal musculature is a dual one, comprising a cortical mechanism for volitional innervation and a subcortical mechanism concerned with integrating facio-vocal activity in emotional expression. Further clinical evidence for this view has been provided by cases of pseudo-bulbar palsy (7), in which bilateral injury to cortico-bulbar connections, with varying degrees of paresis of voluntary movement of the cranial musculature, has been accompanied not by a reduced but by an exaggerated display of mimetico-vocal behavior described as pathological laughter and crying (4). More definite evidence for the subcortical management of facio-vocal activity has come from observation of its retention in arhinencephalic infants (5) and in animals after chronic extirpation of the cortex (2).

Although the facial, vocal and respiratory musculature are innervated by bulbar nuclei, previous work has revealed hypothalamic and midbrain regions of the cat and monkey from which integrated facio-vocal responses could be evoked by electrical stimulation (8). These results suggest that the subcortical mechanism postulated for this activity may be supra-nuclear in position and situated in the rostral portion of the brain stem. The present study has explored this possibility, concerning itself with the effect upon facio-vocal activity of rostral brain stem lesions produced in cats chosen for their normal or exaggerated display of this activity upon nociceptive stimulation or when confronted with other animals.

## METHODS

After varying periods of preoperative examination had established their potentialities for facio-vocal expression, cats were subject to bilateral diencephalic or midbrain lesions, produced, with two exceptions, with the Horsley-Clarke technique. Postoperative examination, with emphasis upon the behavior under discussion, ranged over intervals of one to four months. The extent of the lesion in each case was subsequently determined from serial Weil and thionin sections through the rostral brain stem.

<sup>1</sup> Medical Fellow of the National Research Council.

<sup>2</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

## RESULTS

The series can be divided into two groups: those animals with dien-cephalic or rostral midbrain lesions in which facio-vocal activity remained unimpaired, and those with central midbrain lesions in which this activity was not exhibited after operation.

*Hypothalamic lesions.* Each of two cats in which the hypothalamus was completely destroyed at the mammillary level, thus interrupting all known descending hypothalamic connections, exhibited the somnolence, akinesia and impairment of temperature regulation known to follow such injury. However, pressure of the tail caused these animals to piloerect, circle, strike with the forepaws and bite. Growling, crying and spitting were regularly

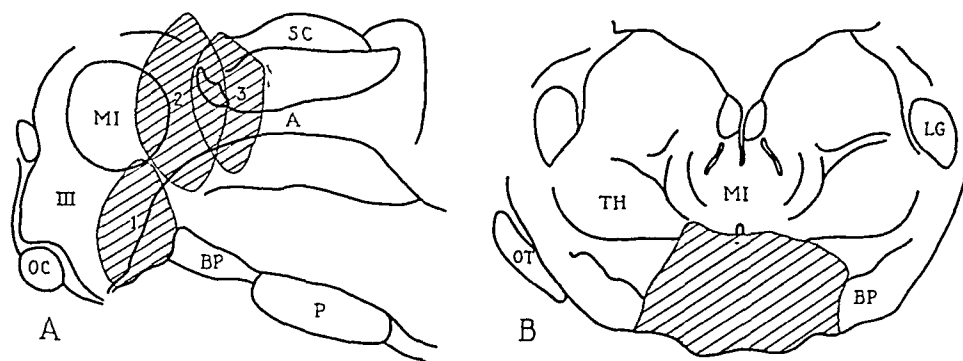


FIG. 1. A. Midsagittal reconstruction of the brain stem upon which is projected the distribution of the lesions in cats 1-3.

B. Cross section through diencephalon showing the greatest extent of the lesion in cat 1.

Abbreviations for all figures are as follows: A—aqueduct, B—brachium pontis, BC—brachium conjunctivum, BIC—brachium of inferior colliculus, BP—basis pedunculi, LG—lateral geniculate body, MG—medial geniculate body, MI—massa intermedia, ML—medial lemniscus, MLF—medial longitudinal fasciculus, OC—optic chiasma, OT—optic tract, P—pons, PC—posterior commissure, SC—superior colliculus, TH—thalamus, III—third ventricle.

evoked by nociceptive stimulation. The response of these animals to barking dogs was slow in onset as compared with that before operation, but included pupillodilatation, retraction of the nictitating membranes and piloerection, all maximal in degree, and retraction of the ears, striking with the forepaws and repeated spitting.

The one of these animals, cat 1, whose lesion is illustrated (Fig. 1A and B), exhibited an unusual display of facio-vocal activity to handling not present before operation. Removal from its cage, taking its rectal temperature, and feeding or cleaning it repeatedly caused growling, spitting and sometimes screaming cries.

Whatever may be the role of the hypothalamus in initiating facio-vocal activity in the intact animal, it is plainly not essential for this behavior.

*Interruption of afferent paths to thalamus.* Because facio-vocal activity

could readily be elicited by cutaneous stimulation in all of the animals of this series before operation, it was thought that interruption of afferent pathways at their ending in the thalamus might indicate whether or not conduction to the diencephalic level was involved in the response.

After operation, cat 2, whose lesion is illustrated in Fig. 1A and 2A, spat at other cats and at dogs, and cried and spat upon nociceptive cutaneous stimulation. Unless some ascending pain pathway other than the lateral spinothalamic tract escaped destruction by these large lesions, this case suggests that afferent conduction can evoke facio-vocal activity below the level of the diencephalon.

*Rostral midbrain lesion.* A third control is provided by cat 3 with the lesion shown in Figs. 1A and 2B, in which facio-vocal activity was not impaired after destruction of the pretectal region, central grey and adjacent tegmentum at the rostral end of the midbrain.

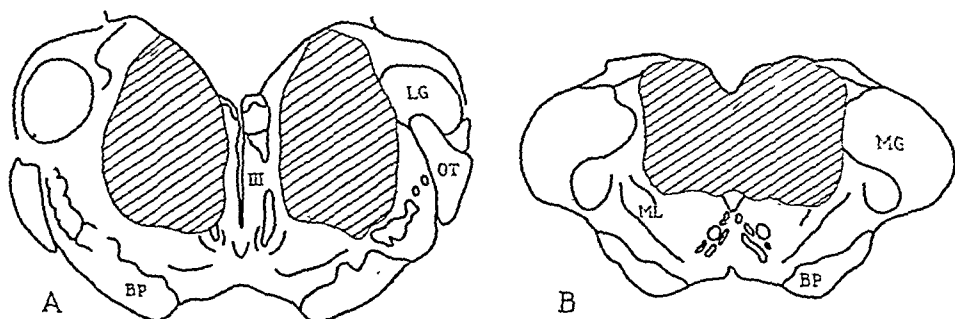


FIG. 2. Cross sections through midbrain showing the greatest extent of the lesions in cats 2(A) and 3(B).

*Transection of the midbrain-diencephalic junction.* In each of the preceding animals a circumscribed region of possible importance for facio-vocal activity was destroyed bilaterally. Postoperative maintenance of this activity in each case might be ascribed to preservation of neighboring structures. That this was not the case is demonstrated by cat 4, which was observed for one month following transection of the midbrain-diencephalic junction (Fig. 3A).

No vocalization could be elicited in this animal during the first postoperative week, but thereafter growling, spitting and crying, the latter varying in intensity from chirruping meows to yowls, could regularly be induced by nociceptive stimulation and often simply by handling. Piloerection frequently accompanied this facio-vocal activity. Gross motor behavior was lacking for, though the animal could get to its feet and, with some steadying, maintain the standing posture for short periods, it did this only immediately preceding defecation.

The easily induced, integrated facio-vocal activity exhibited by this animal, and comparing favorably with the normal in variety, indicated that this behavior is managed by the brain stem below the diencephalon.

*Excitable midbrain area for facio-vocal activity.* Attention was then focussed upon the midbrain and in placing lesions in this part of the brain



stem, cognizance was taken of the results of earlier work in the cat that had demonstrated an excitable area from which repetitive crying and spitting could be evoked by electrical stimulation (8). The localization data were

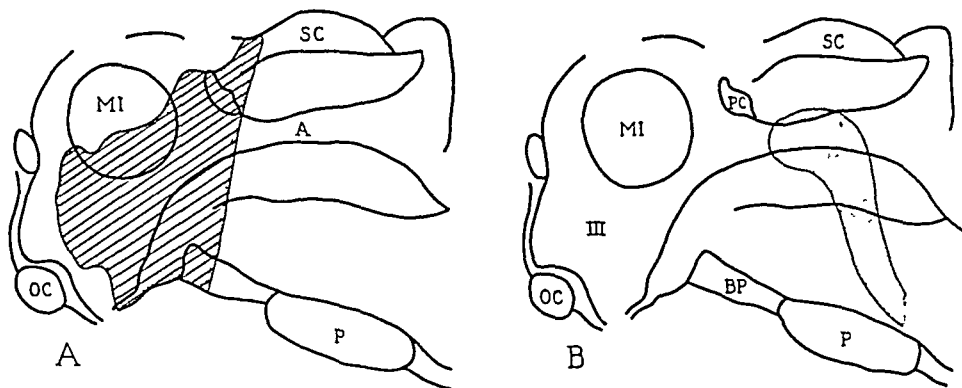


FIG. 3. A. Midsagittal reconstruction of the brain stem upon which is projected the distribution of the transection in cat 4. The brain stem was completely divided except for the lateral portion of the right medial geniculate body.

B. Midsagittal reconstruction of the brain stem, upon which has been indicated in stipple the area yielding facio-vocal responses to electrical stimulation. (Data from Magoun, Atlas, Ingersoll and Ranson, 8.)

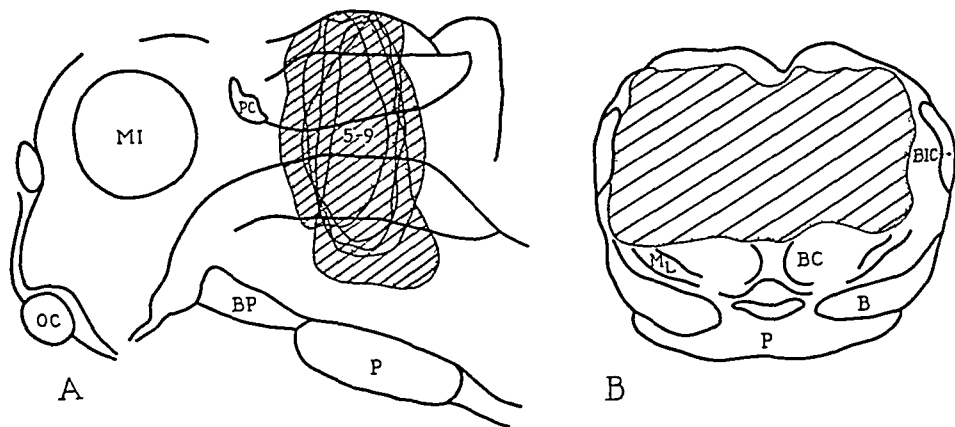


FIG. 4. A. Midsagittal reconstruction of the brain stem upon which is projected the distribution of the lesions in cats 5-9.

B. Cross section through the midbrain showing the greatest extent of the lesion in cat 5.

reviewed and the excitable region projected in stipple upon a reconstruction of the midsagittal plane (Fig. 3B). In its rostral part this region comprised the central grey around the aqueduct and the adjacent midbrain tegmentum.

*Central midbrain lesions.* In each of five cats lesions were produced destroying much of this excitable area bilaterally (Fig. 4A). Some vocaliza-

tion was evoked during the production of the lesion, but after operation, though the animals were repeatedly subjected to conditions that should have provoked it, facio-vocal activity was never subsequently elicited in two cases and only isolated instances of it were exhibited by the other three. Following are brief protocols of these animals:

*Cat 5.* A large, wild male had been in the laboratory a month and every time it was approached crouched in the back of its cage, head retracted, ears flattened, hair erected, repeatedly growling and spitting. It was extremely difficult to handle this animal because of its ferocious behavior. While it exhibited some antipathy and facio-vocal behavior to dogs, most of it was directed at man.

During the first week after the production of the large midbrain lesion shown in Fig. 4B, the cat appeared comatose and except for a few progression movements when handled or fed, showed no activity. Transient accessions of flaccidity were encountered during which all tone was lost, and though respiration continued, the knee jerk became spinal in type (6).

In its subsequent course the animal regained the ability to walk and even run, with hypermetria in the steps of the forelegs, and to take food with lunging, ill directed bites, gulping it without chewing. When out of its cage, the cat usually wandered aimlessly about, frequently sniffing, and if any object was put just before its face, clutched and bit it, possibly with the impression that it was food, for its appetite was voracious.

Although repeatedly confronted with dogs, attacked by other cats and subject to extreme procedures by observers, during its survival of 2 months after operation, this animal never exhibited any aggressive or defensive behavior, never piloerected, never uttered a sound or displayed any mimetic activity, never in fact showed any response, except to urinate or sometimes to defecate.

*Cat 6.* A wild male had been in the laboratory several weeks, spitting whenever anyone looked into its cage, and attacking whenever one tried to handle it. It flattened its ears, hissed and piloerected when confronted with dogs, but exhibited more aggressive behavior to man than to other animals.

This cat recovered rapidly after production of the smaller midbrain lesion shown in Fig. 5A. It ate well from the 3rd postoperative day and showed no impairment in gait or activity after the first week. Through its survival of 2 months, it would struggle violently and lash its tail when held up to dogs and run away and jump into its cage when released, and would bite and struggle vigorously to escape when subjected to nociceptive stimulation, but never piloerected<sup>1</sup> or exhibited any facio-vocal activity whatever.

*Cat 7.* This large male was apprehensive in the laboratory but was friendly when petted. It objected loudly, with growling cries and spitting, to nociceptive stimulation, and when held up to dogs, struck, spat, piloerected fully and tried to escape.

During a survival of 4 months after the lesion shown in Fig. 5B, this animal tried vigorously to escape and urinated whenever confronted with dogs, but never piloerected or exhibited any facio-vocal activity. Nociceptive stimulation caused clawing, biting, lashing of the tail and some piloerection, but never vocalization or grimacing. While this animal never uttered a sound after operation in the waking state, terminal strong electrical stimulation of the sciatic nerve under light nembutal anesthesia caused groaning.<sup>4</sup>

<sup>1</sup> It is noteworthy that piloerection could still be evoked by nociceptive stimulation or the presence of dogs after destruction of the caudal hypothalamus in cats 1 and 2 and often appeared simply to handling in cat 4 with transection of the diencephalic-midbrain junction. The failure of piloerection to be elicited by these situations in cats 5 and 6 with central midbrain lesions points to the importance of mesencephalic structures in initiating piloerection in emotional excitement. This is in contrast to the initiation of piloerection in response to cold, which like other thermoregulatory functions is dependent upon the integrity of the hypothalamus (10).

<sup>4</sup> Terminal stimulation of the sciatic under light anesthesia was tested in all five animals in this group (cats 5-9). This was the only instance in which a vocal response resulted.

*Cat 8.* This large friendly male responded to petting by rubbing its head against the observer and purring, but objected to nociceptive stimulation with growling cries, spitting, clawing and struggling, and when confronted with dogs, struck, piloerected and spat repeatedly.

Five days after the production of the lesion shown in Fig. 6A the cat growled when his bladder was expressed.<sup>5</sup> A month after operation, when confined in a cat box with head protruding, he spat once. Except on these two occasions, repeated exposure to dogs, nociceptive stimulation or confinement, during a period of four and one half months, while it frequently caused struggling, biting and attempts to escape, was always without results in evoking facio-vocal activity. This animal continued to respond to petting after

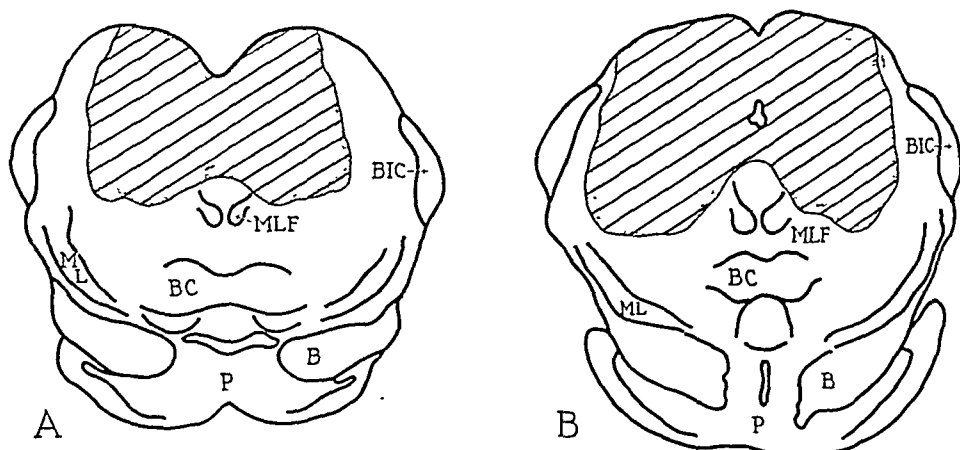


FIG. 5. Cross sections through the midbrain showing the greatest extent of the lesions in cats 6(A) and 7(B).

operation—a vibration of the larynx could frequently be detected on palpation, and on a few occasions a low purring could be heard.

*Cat 9.* A large friendly male meowed, purred and rubbed its head against the observer when petted. It growled, cried and spat loudly to pressure of the skin, and when held to dogs it struck, flattened its ears, piloerected and spat repeatedly while trying to escape.

The lesion produced is shown in Fig. 6B. Evacuation of the bladder during the first two postoperative weeks frequently elicited a low rumbling, resembling purring. Purring could later sometimes be induced by petting. Repeated attempts to evoke facio-vocal activity by nociceptive stimulation and exposure to dogs during a 3-month period after operation were all without result, though struggling, striking, lashing of the tail, some piloerection and urination could be induced.

On the day preceding sacrifice, the animal was warmed with an electric heater to determine whether panting could be elicited.<sup>6</sup> Shortly before panting began, petting the animal caused it to spit, and with the onset of panting, spitting was frequent both when the cat was unmolested and when its rectal temperature was taken. On its terminal day the animal spat twice when its head was fixed in a Czermak holder under light nembutal anesthesia. This isolated appearance of spitting during panting and manipulation of the oral region appeared to involve a summation process.

<sup>5</sup> All the cats of this group (Nos. 5–9) required manual evacuation of the bladder during the early postoperative period.

<sup>6</sup> Three other cats (Nos. 6, 7 and 8) in this group were similarly tested with a heater. Panting was elicited in all three, but without any vocal activity.

In two of the animals of this group, then, no facio-vocal activity could ever be elicited during periods of 2 months after operation. In a third a single instance of groaning to direct afferent nerve stimulation was the only vocalization in a period of 4 months. In the remaining two cases growling and purring and isolated instances of spitting were exhibited, but did not obscure the fact that facio-vocal activity was extraordinarily reduced from its preoperative incidence. These five animals indicate that within the area of the midbrain destroyed is a mechanism of major importance for the integration of facio-vocal activity in emotional expression.

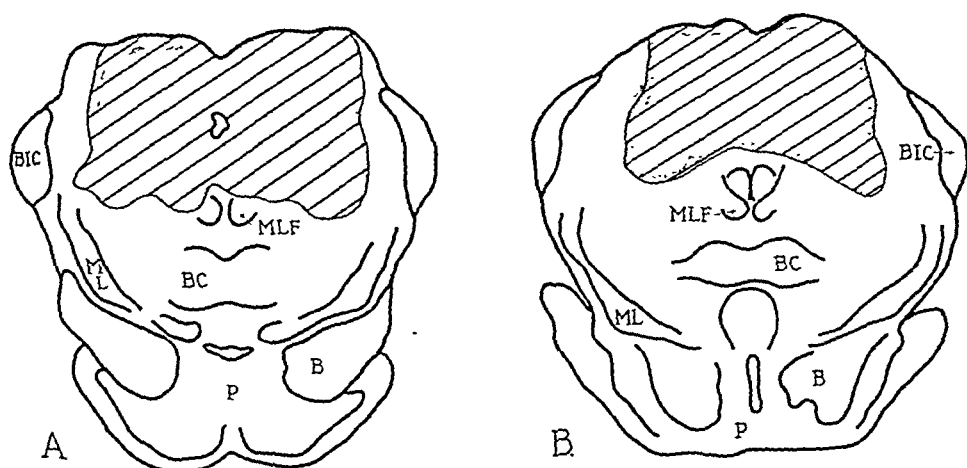


FIG. 6. Cross sections through the midbrain showing the greatest extent of the lesions in cats 8(A) and 9(B).

*Tectumectomy.* In each of the cases just discussed, injury to a considerable part of the tectum of the superior colliculus was also present, and the question might be raised whether tectal injury or whether injury to central grey and tegmentum was the factor responsible for impairment in facio-vocal activity.

A female animal was, therefore, observed for 2 weeks after aspiration of the tectum of the superior colliculus. This cat meowed frequently from the first postoperative day, cried loudly on nociceptive stimulation and, when confronted with dogs, piloerected and cried, but did not spit, as it had done before operation. The results from this animal make it evident that failure of vocalization in the animals described above cannot be attributed to injury to the superior colliculus. The absence of spitting in this case can be explained either by destruction of the tectum or by injury to the subjacent midbrain, for the lesion extended ventrally to a line drawn through the top of the aqueduct.

### DISCUSSION

These results point to the general conclusion that the central part of the midbrain—the aqueductal grey and adjacent tegmentum beneath the superior colliculus—contains structures of importance for integrating facio-vocal behavior in emotional expression. The loss or reduction of this behavior

that follows injury to this region does not appear to be the result of interrupting afferent pathways to higher levels for after their interruption at the midbrain-diencephalic junction, facio-vocal behavior could still be evoked. Similarly it does not seem possible to explain the facio-vocal deficit after central midbrain lesions as resulting from the interruption of descending pathways from higher levels which simply pass through this area, for this behavior could still be elicited after lesions of the hypothalamus and rostral midbrain and after transection of the brain stem at the midbrain-diencephalic junction. The facio-vocal deficit that follows central midbrain lesions appears to be attributable, therefore, to injury to intrinsic structures in this region.

It is not evident from the present results whether injury to the central grey or to the adjacent tegmentum is the more important in the resulting loss of facio-vocal behavior. Loss of this behavior after destruction limited to the periaqueductal grey in the cat (no. 1) suggests the predominant importance of the latter structure. If this is the case, facio-vocal responses evoked from stimulation of the midbrain tegmentum (cat 8) can perhaps be attributed to excitation of either afferent connections to or efferent connections from the periaqueductal grey matter.

It should be noted that these animals with transverse lesions of the central grey and adjacent tegmentum (cats 3 and 6 to 9) did not exhibit the postoperative akinesia and loss of consciousness noted by Bailey and Davis (1) after extensive, antero-posterior lesions of the periaqueductal grey matter. One animal of the present series (cat 5), in which a larger amount of the adjacent tegmentum was destroyed than usual, was akinetic and comatose after operation, but only during the first postoperative week.

The facio-vocal deficit which five of the present specimens exhibited (cats 5 to 9) should not be attributed, therefore, to any general reduction of the animals' behavior. The specificity of their deficit was further emphasized by the maintenance after operation of other activities involving the oral region, for reflex movements and biting, chewing, lapping and panting were not prevented by these lesions.

The midbrain facio-vocal mechanism to which these experiments point appears to be concerned more with expressions of anger or complaint than with those of pleasure or contentment, for purring appeared more readily elicitable in two of the cats of the series than did growling, crying or spitting. It may be remarked that the deficit which the cats exhibited in anger or complaint consisted of an absence of facio-vocal behavior, not of an impairment or incoordination of its execution. Furthermore, in the two animals in which isolated instances of spitting were encountered, this complicated act was performed perfectly. This suggests that the midbrain facio-vocal mechanism is not concerned with integrating the sequence of activities in the component bulbar nuclei innervating the cranial and respiratory musculature during vocalization—which probably is managed by interneurons at the bulbar level—but rather is concerned with precipitating their integrated

performance at times when it forms an appropriate part of the behavior of the animal as a whole.

### SUMMARY

Central midbrain lesions, destroying the periaqueductal grey matter and adjacent tegmentum beneath the superior colliculus, abolished or greatly reduced facio-vocal behavior in a series of cats. The maintenance of other activities in these animals and the preservation of facio-vocal behavior in other animals after control lesions elsewhere in the rostral brain stem emphasized the specificity of the deficit. Facio-vocal responses had previously been elicited by electrical stimulation within the mesencephalic region destroyed in these experiments. The two lines of evidence point to a central midbrain mechanism for integrating facio-vocal behavior in emotional expression.

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# CENTRAL EFFECTS OF CENTRIPETAL IMPULSES IN AXONS OF SPINAL VENTRAL ROOTS<sup>1</sup>

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## INTRODUCTION

THE EXPERIMENTS of Müller (18) and others demonstrated that stimulation of the central end of a severed ventral spinal root does not result in the production of muscular movements; that is to say, an antidromic volley of impulses in a group of motor axons does not result in the discharge of other motoneurons. However, such antidromic volleys are not without effect on the spinal cord. It is well known that they condition reflex discharges of the attached motoneurons (9, 10, 13). Three additional phenomena have recently been demonstrated: (i) the retrograde axonal impulses spread over the somas of the attached motoneurons, producing action currents (16, 17, 21); (ii) centripetal impulses are then set up, after a brief latency, in a fraction of the stimulated motoneurons (20); and (iii) tested two-neuron arc reflex discharges into groups of motor axons other than those occupied by the antidromic volley are conditioned, *i.e.*, facilitated or inhibited as the case may be (20).

The discovery of the latter phenomenon has prompted a search for evidences of neural activity in the cord during the period subsequent to the arrival of a volley of impulses travelling centripetally in axons of ventral roots. Recordings made with micro-electrodes have now revealed relatively prolonged bursts of action potentials, which are believed to originate in interneurons of the ventral horn. Although this activity has not been exhaustively investigated, information concerning it is presented at this time because of its possible importance in the economy of the ventral gray matter and, more particularly, because the pattern of discharge of the individual neurons is sufficiently unusual to be of general interest.

## METHODS

The experiments were carried out on cats and rabbits that had been either decerebrated or lightly narcotized with pentobarbital sodium ("Nembutal"). After a laminectomy had been performed and the necessary spinal roots cut, the cord was covered with a layer of paraffin oil. Centripetal volleys were initiated in groups of motor axons either by stimulation of ventral roots which had been severed as they passed through the dura mater or by stimulation of the central ends of cut peripheral motor nerves. In the latter case it was necessary to sever ipsilateral dorsal roots to prevent entrance into the cord of impulses in sensory fibers of the stimulated nerve. Records were made of the potential differences which arose between a micro-electrode carefully inserted into the spinal cord

<sup>1</sup> A brief account of these observations was presented in 1942 at the Boston meetings of the American Physiological Society (see *Fed. Proc.*, 1942, 1: 71).

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and a second, indifferently placed electrode. The micro-electrodes were steel needles of shank diameter 50-100 micra. They were insulated with enamel except at their tips, which had been ground at a gradual taper to sharp points. The usual amplifier oscillograph, and stimulating apparatus were used.

## RESULTS

1. *Initiation of spike activity in the ventral horn by antidromic motor volleys.* A volley of centripetal impulses which enters the spinal cord over the alpha fibers of a ventral root initiates prolonged spike activity in the ventral horn. The illustrative record of Fig. 1 was obtained during an experiment on a decerebrated cat. The centripetal motor volley was evoked in the axons of the seventh lumbar ( $L_7$ ) ventral root by application of an alpha-strength

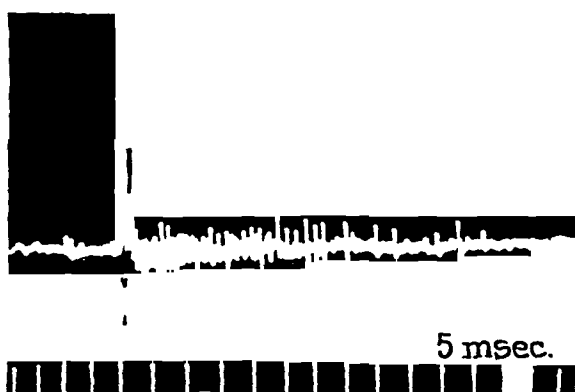


FIG. 1. Action potentials developed in the  $L_7$  ventral horn of a decerebrated cat as a sequel to the application to the ipsilateral  $L_7$  ventral root of a single shock maximal for alpha fibers. In this and all subsequent figures the electrical changes were recorded between a micro-electrode in the ventral horn and a second, indifferently placed electrode; negativity at the micro-electrode is represented by an upward deflection.

shock. Inasmuch as the root had been severed intradurally at a point distal to the stimulating electrodes, there was no possibility that impulses in fibers entering the cord via the dorsal roots were initiated either directly or indirectly. The oscillogram shows the potential changes which arose between a micro-electrode in the ipsilateral  $L_7$  ventral horn and a second electrode placed at a distant (indifferent) point on the preparation. It is seen that the centripetal volley evoked a burst of action potentials which persisted, in progressively decreasing numbers, for about 50 msec. Similar activity in the ventral horn of the  $L_6$  or  $L_7$  segments may be evoked by stimulation in the hind limb of peripherally severed motor nerves (e.g., the sciatic, hamstring, and crural nerves), even when the cord has been transected at the upper lumbar and midsacral levels and all intervening ipsilateral dorsal roots severed intradurally. In all cases the action potentials have been recorded only from the ipsilateral ventral horn at and near the level of entry of the

stimulated ventral root. Similar activity has not been observed in other parts of the spinal cord and brain stem.

It may be noted that the positions of the recording micro-electrodes in the cord have not been confirmed in histological sections. However, the assertion that the responding units in question are located in the ventral horn is not based merely on the fact that their action potentials may be recorded only when an electrode is advanced an appropriate distance ventrad of the dorsal surface of the cord. The simultaneous recording at these posi-

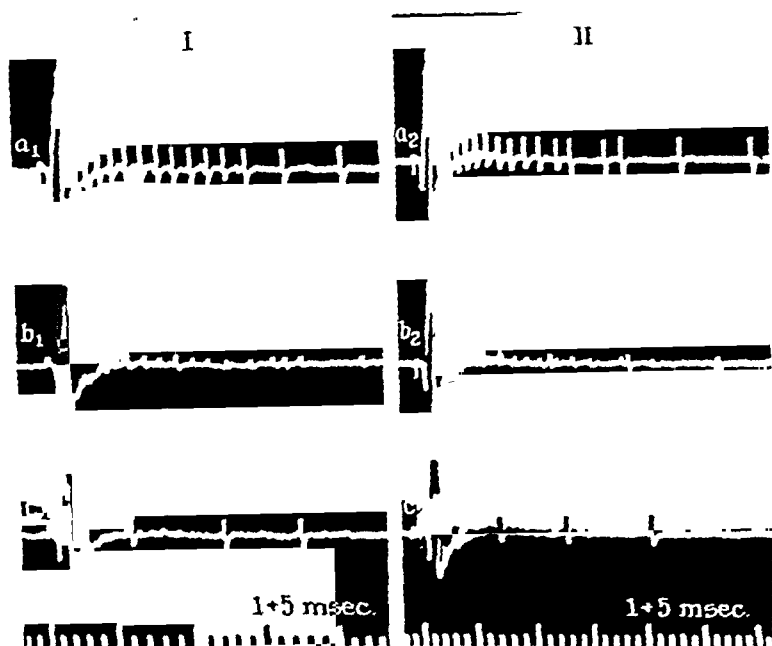


FIG. 2. Action potentials of single neurons in the ventral horn. The stimulus was a centripetal volley in the motor fibers of the hamstring nerve, the caudal cord having been transected and all ipsilateral dorsal roots caudad of L<sub>7</sub> severed. Records *a* were taken at a position in the ventral horn, records *c* after advancing the electrode 0.15 mm ventrally; records *b* were taken at an intermediate position. The records of Column I were obtained within a few minutes after initially inserting the micro-electrode; those of Column II, 30–40 minutes later. Rabbit anesthetized with pentobarbital sodium.

tions of large action potentials due to the antidromically stimulated motoneuronal somas adds convincing evidence of the location of the electrode within the ventral horn (see below).

2. *Identification of the active neurons.* Careful insertion of small micro-electrodes into the ventral horn makes it possible to record in effective isolation the action potentials of single units which participate in the discharges illustrated by Fig. 1. Figure 2a<sub>1</sub> shows the action potentials of such a unit in the ventral horn of a rabbit. The artefact signalled the delivery of an alpha-strength shock to the deafferented hamstring nerve. The approach of

the centripetal volley in the motor axons was heralded by the initial positive (downward) deflection, and its arrival at the motoneuronal somas by the major negative deflection (cf. 16, 17, 21). There followed a series of about 15 action potentials (spikes) which appeared in a regular sequence of progressively declining frequency.

There can be no reasonable doubt that such series of action potentials represent the repetitive discharges of single neurons located in the ventral horn. The successive action potentials in a series are relatively constant in size and shape; they succeed each other in a regular series; and the individual spikes are of brief duration and small size (*i.e.*, *ca.* 100–200  $\mu\text{v.}$ ). Furthermore, any particular series can be recorded only from a very restricted region within the ventral horn. In the illustrative experiment from which Fig. 2 is derived, an antidromic hamstring volley—the ipsilateral dorsal roots had been severed—caused a neuron in the ventral horn to discharge about 15 times (records *a*). When the micro-electrode was inserted only 0.15 mm. (*i.e.*, 150 micra) deeper, as is shown in records *c*, the discharges of the neuron were no longer apparent at the amplification employed but the action potentials of a second neuron, which discharged 4–5 times, were recorded. At a position intermediate between the two points (records *b*), small potential changes indicated the activity of both neurons and perhaps of others as well.

Current concepts suggest that the repetitive action potentials may be related to the somas of neurons, and offer three more or less plausible alternative explanations for their origin. They might be: (i) a series of impulses originating in some unknown manner at the peripheral cut end of the central stump of the stimulated motor nerve and travelling antidromically over ventral root axons into the attached motoneuronal somas; (ii) the action potentials of motoneurons discharged repetitively as a sequel to the arrival at the cord of the single antidromic volley in the axons of the same or other motoneurons; or (iii) the action potentials of interneurons discharged in the ventral horn as a consequence of the antidromic volley and its central sequelae.

Explanation (i) is rendered implausible by the fact that, in contrast with the potential changes produced by the antidromically stimulated pool of motoneurons, the negative phase of each repetitive action potential usually is not preceded by a positive deflection such as must herald the approach of an impulse from a distance. Moreover, inasmuch as the neurons frequently can be fired by the stimulation of any one of two or more peripheral motor nerves (see below), adoption of explanation (i) would entail the subsidiary assumption that at least some of the hypothetical motoneurons must send branches of their axons into each of the different peripheral nerves.

Evidence which casts doubt on explanation (ii) is also available. No known discharges of motoneurons occur at the high frequencies which characterize the units under consideration (cf. 3, 4, 19). Moreover, careful search has failed to reveal that centripetal impulses in a group of ventral root axons evoke centrifugal impulses in neighboring motor axons, and the

few centrifugal impulses which do appear in the axons of a small percentage of a group of antidromically-stimulated motoneurons emerge from the cord after a central latency of only about 0.8–1+ msec. (20). The ventral horn activity under consideration, however, typically persists for 30–50 msec. after the arrival of the antidromic volley. Finally, assumption that the action potentials represent the repetitive discharges of a fraction of the *same* motoneurons which are occupied by the conditioning volley would also require the subsidiary postulate mentioned above in the discussion of explanation (i).

Thus exclusion of explanations (i) and (ii), and of additional less plausible possibilities, permits the provisional assignment of the action potentials to repetitively discharging *interneurons* located in the ventral horn. This interpretation will be assumed in what follows.

Anatomical studies (cf. 7) do in fact reveal the existence of numerous, relatively small interneurons of several types scattered through the ventral horn. It would be premature to attempt to identify the interneurons activated by an antidromic motor volley with one or more of the specific types described by anatomists. However, it may be noted that the neurons now under discussion appear to occur in various parts of the ipsilateral ventral horn, and that no evidences of spike activity in the contralateral ventral horn, or change in the thresholds of contralaterally located motoneurons, have been observed. Nor has success attended initial attempts to find, subsequent to an antidromic motor volley, impulses in Gower's tract—an experiment prompted by the possibility that the activated interneurons might include the "border cells" of Cooper and Sherrington (8).

3. *Regulation of the discharges of the neurons.* a. *Effect of the size of an antidromic volley.* As the size of an antidromic volley is increased, a ventral horn interneuron typically responds with an increasing number of discharges (action potentials) at increasing frequencies, and the latency of the first impulse progressively decreases. The illustrative records of Fig. 3, taken at two sweep speeds, show the responses of such an interneuron to centripetal volleys of increasing size (records a to g) in the deafferented sciatic nerve of a rabbit. As is shown in record g, and also in Figs. 2a and 4c, large (maximal) antidromic volleys may be followed by a series of as many as 10–15 or more discharges during a period of ca. 30 msec. The intervals at which the action potentials succeed one another increase progressively along a course which is approximately logarithmic except at the beginning of the discharges of highest frequency; in such cases (e.g., Fig. 3g) the intervals between the first few action potentials remain nearly constant at a minimum of ca. 0.6–0.7 msec. (i.e., the frequency is ca. 1500 per sec.). This observation demonstrates that the absolute refractory period of the interneurons is no longer than 0.6–0.7 msec. Apparently the intensity of the stimulation is often so great that the frequency of discharge is determined by the rapid change in excitability which presumably characterizes the earliest part of the relative refractory period.

The time of arrival of the centripetal volley at the motoneuronal somas is signalled by the rise of the oscillograph spot from the initial positive trough due to the approaching ventral root impulses into the negativity referable to the motoneuronal somas. Measured from this time, as in records  $f_2$  and  $g_2$  of Fig. 3, the minimal latency for the first interneuronal action

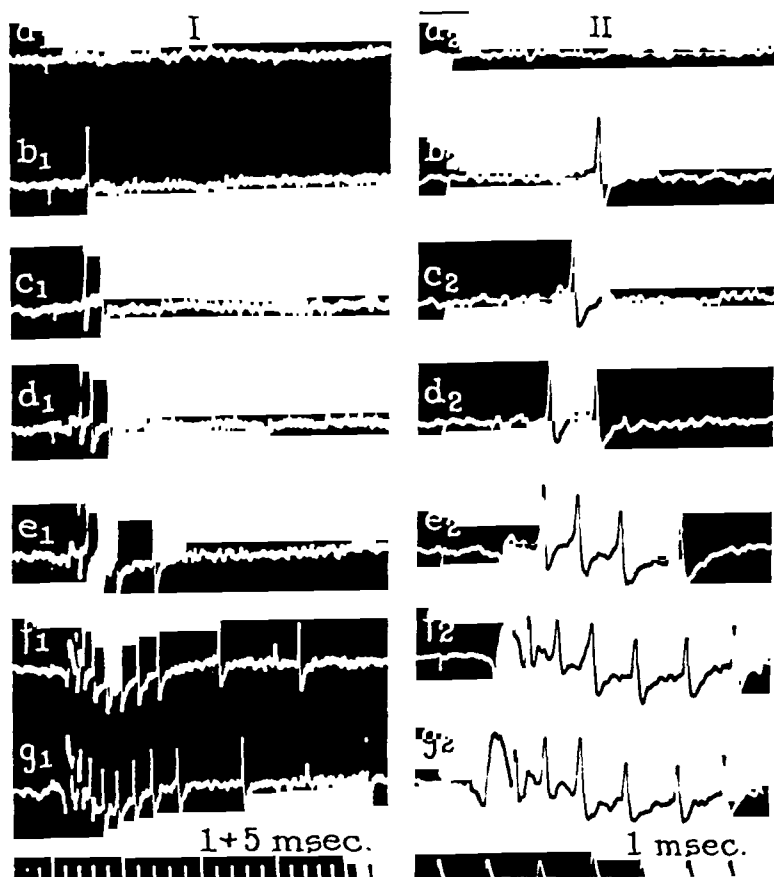


FIG. 3. Responses of motoneuronal somas and of a ventral horn interneuron to single antidromic motor volleys of increasing size (records  $a$  to  $g$ ) in the motor fibers of the sciatic nerve. Column I, relatively slow time scale; Column II, faster time scale. Rabbit anesthetized with pentobarbital sodium.

potential set up by a maximal antidromic motor volley proves to be 0.6–0.7 msec.—a duration equal to that of a single synaptic delay (14, 16). In no instances have impulses been observed to arise after significantly shorter latencies.

In the experiment illustrated by Fig. 3, as the centripetal volley was made progressively smaller, the latency of the first interneuronal action potential increased gradually by more than a millisecond (compare records  $b_2$  and  $g_2$ ).

Only a part of the increased latency may reasonably be assigned to an increased delay in the time of arrival at the ventral horn of the small volley of antidromic impulses set up in the motor axons by the weaker shock.

*b. Conditioning by ventral root volleys.* Impulses in many ventral root axons regulate the discharges of individual interneurons of the type under discussion (cf. Fig. 3). Summation and inhibition may be revealed by the

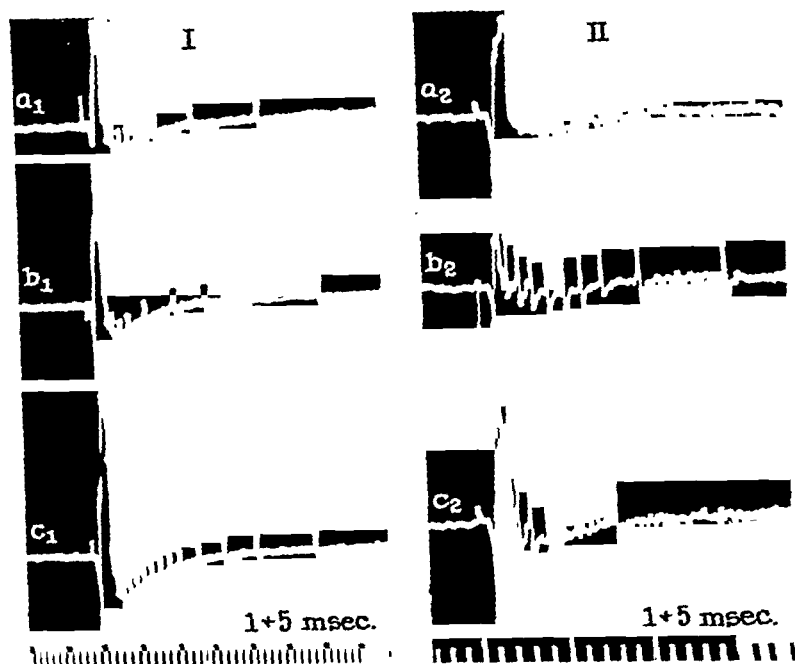


FIG. 4. Summation and inhibition of the discharges of single interneurons of the ventral horn as revealed by the use of essentially simultaneous volleys in two different deafferented motor nerves. Column I: records from an experiment on an anesthetized rabbit with severed ipsilateral dorsal roots;  $a_1$ , stimulation of the sciatic (peroneal plus tibial) nerve;  $b_1$ , stimulation of the hamstring nerve;  $c_1$ , simultaneous stimulation of both nerves. Column II: records from another experiment on an anesthetized rabbit with severed ipsilateral dorsal roots;  $a_2$ , stimulation of one branch of the hamstring nerve;  $b_2$ , stimulation of a second branch of the hamstring nerve;  $c_2$ , simultaneous stimulation of both branches.

use of centripetal volleys in two different deafferented nerves. The records of column I of Fig. 4 reveal summation. Record  $a_1$  shows the effect of stimulation of the sciatic (peroneal and tibial) motor fibers; record  $b_1$  shows the response to stimulation of the deafferented hamstring nerve. Combination of both centripetal motor volleys (record  $c_1$ ) resulted in more discharges of the neuron in question than were produced by either volley in isolation. Inhibition is illustrated by the records of column II. The neuron near the micro-electrode did not discharge subsequent to the arrival at the cord of a centripetal volley in one branch of the deafferented hamstring nerve (record

$a_2$ ). It responded 9 times following an antidromic volley in another branch of the hamstring nerve (record  $b_2$ ). As shown in record  $c_2$ , simultaneous stimulation of the motor fibers of both branches was followed by only five discharges.

The use of two successive centripetal motor volleys demonstrates that the internuncial system is conditioned by antecedent activity. Records  $a-c$

of Fig. 5 reveal a reduction in the duration of the period of interneuronal discharge following the second of two successive antidromic motor volleys. Records  $d-f$  reveal in addition a reduction in the initial frequencies of the discharge consequent upon the second of two such volleys. Thus the frequencies at which the action potentials succeed each other, the number of action potentials, and the duration of the period of discharge are all diminished as a consequence of preceding activity.

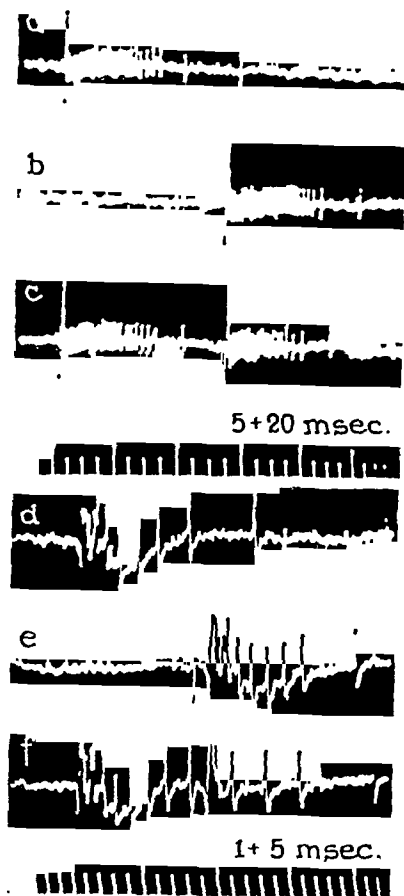


FIG. 5. Conditioning of interneuronal discharges evoked by the second of two successive centripetal motor volleys. Records  $a-c$  from an experiment on an anesthetized cat upon stimulation of the hamstring nerve. Records  $d-f$  from an experiment on an anesthetized rabbit upon stimulation of the sciatic nerve 66 min. after insertion of the micro-electrode. In both experiments the ipsilateral dorsal roots had been severed.

#### *c. Conditioning by dorsal root volleys.*

Ventral horn interneurons which discharge repetitively as a sequel to large antidromic volleys frequently are not thrown into activity by dorsal root volleys, at least when the latter suffice to initiate discharges of relatively few motoneurons (cf. Fig. 6). In other instances, however, a dorsal root volley is followed by discharges of neurons in the ventral horn (Fig. 7; cf. also 12); in some cases some of these neurons apparently are identical with interneurons fired by an antidromic motor volley.

In several experiments the central effects of a dorsal root volley did not significantly alter the pattern of the interneuronal discharge which followed an antidromic volley arriving at the cord 10-20 msec. subsequently (Fig. 6, Fig. 7  $b$  and  $d$ ). It is of interest that in the experiment illustrated by Fig. 6 the antecedent dorsal root volley exerted no pronounced conditioning effect

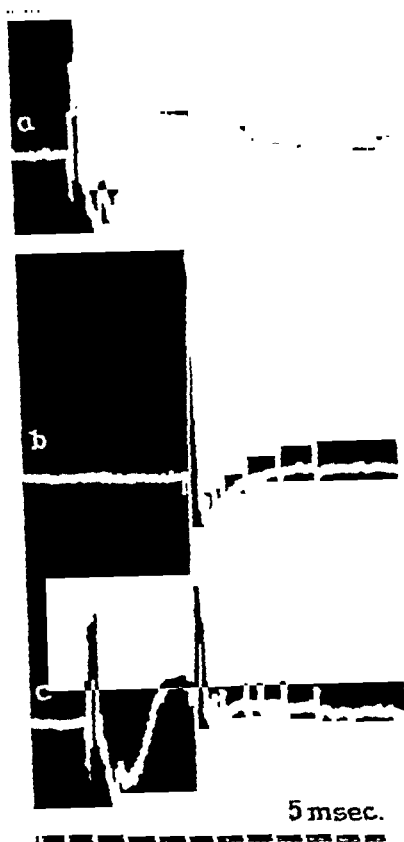


FIG. 6. Effect of a conditioning dorsal root volley on the responses of an interneuron in the ventral horn to an antidromic motor volley. Record *a*, stimulation of the  $L_7$  dorsal root fibers; *b*, stimulation of the deafferented sciatic nerve; *c*, stimulation of the  $L_7$  dorsal root fibers followed by stimulation of the sciatic nerve. Note that the pattern of discharge of the interneuron was not appreciably affected by the conditioning dorsal root volley even though the response of the motoneuronal somas to the centripetal volley of impulses in their axons was considerably modified (cf. 21). From an experiment on an anesthetized rabbit with severed ipsilateral dorsal roots.

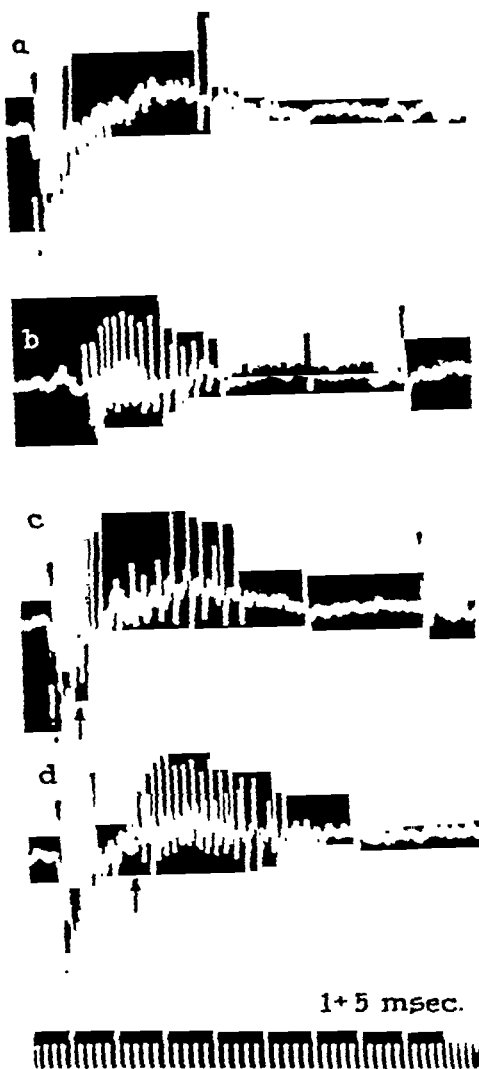


FIG. 7. Excitation of ventral horn interneurons by primary afferent and antidromic motor volleys. Record *a*, stimulation of  $L_1$  dorsal root; *b*, stimulation of the deafferented crural nerve; *c* and *d*, stimulation of  $L_1$  dorsal root followed by stimulation of the deafferented crural nerve. Note the presence of a marked conditioning effect in record *c* and the absence of such a definite effect in record *d*. From an experiment on an anesthetized cat with severed ipsilateral dorsal roots.



on the interneuronal discharge even though it significantly altered the response of the motoneuronal somas to the antidromic volley (cf. 21). In one experiment (cf. Fig. 7 *b* and *c*) a conditioning dorsal root volley preceding an antidromic volley by 4–5 msec. did alter the pattern of the interneuronal discharge consequent upon the antidromic volley; the significant feature of the conditioning was the introduction of a silent period during the early part of the interneuronal discharge.

4. *Evaluation of the possible role of injury.* It is important to ask whether injury caused by the introduction into the cord of the recording micro-electrode is a factor determining or influencing the character of the interneuronal discharges consequent upon an antidromic volley in ventral root fibers. The possible participation of injury has not been emphasized in other instances in which discharges of single neurons in the spinal cord have been carefully studied and found to be in accord with physiological inferences based on anatomical knowledge (11, 12). The possibility of a role of injury in the case of the activity described in this paper comes more prominently to mind, however, principally because of the strikingly high frequencies at which the units discharge.

The following observations supply evidence that the discharges are not dependent upon injury. (i) The pattern of the discharges of an interneuron that is thrown into repetitive activity by an antidromic motor volley frequently remains unaltered during prolonged periods of time beginning shortly after the insertion of the recording micro-electrode, whereas spontaneous high-frequency and other discharges known to be due to injury usually change rapidly with the passage of time (cf. 1, 5, 23). The constancy of the pattern of discharge of an interneuron is illustrated by the records of Fig. 2. The records of column I were taken shortly after the insertion of a recording micro-electrode into the cord, those of column II forty minutes later. It is seen that no significant change had occurred during this interval. Other unitary discharges have been observed with similar results for considerably longer periods of time. (ii) As a micro-electrode is inserted progressively deeper into the ventral horn, the pattern of the discharges of an interneuron at the first position at which it is observable is the same as at positions slightly deeper (*i.e.*, more ventral) within the gray matter, even though the size of the recorded action potentials may be considerably larger at one of the latter positions. (iii) As stated above, the minimum latency for the first of the interneuronal action potentials which follow an antidromic motor volley is 0.6–0.7 msec.—the duration of a single synaptic delay—whereas significantly shorter latencies are known to characterize cross-excitation in injured axons (23). (iv) The recorded action potentials usually have a brief but prominent negative phase, whereas records of impulses approaching admittedly injured regions of axons in volume conductors are recorded as relatively prolonged positive deflections (24).

## DISCUSSION

Two points concerning the repetitive action potentials which have been described above and tentatively attributed to interneurons in the ventral horn invite discussion. These are (i) the possible role of the activity in the normal functioning of the spinal cord, and (ii) the significance of the high frequency and general pattern of the discharges.

At the outset it may be stated that the processes initiating the excitation of the interneurons must depend on one or both of the following events: (i) the arrival of impulses at the terminal knobs of the recurrent collaterals with which some motoneurons are supplied (cf. 7) or of hypothetical afferent fibers in the ventral roots; or (ii) the setting up of environmental changes by the activity of the antidromically stimulated motoneuronal somas. Explanation in terms of (i) must remain speculative in the absence of exact knowledge concerning the terminations of the recurrent collaterals of the motoneurons. The alternative mechanism (ii), which would not depend on specific fiber connections, comes to mind because of lack of information concerning the recurrent collaterals (sometimes stated to be few in number), and because of the large action currents produced by impulses in the motoneuronal somas. The significant question is whether these currents, as well as chemical diffusion gradients which might be set up in time to affect the interneurons during at least the later part of their period of discharge, are sufficiently large to exert quantitatively important effects on the excitability of the near-by interneurons. There is no definitive evidence that this is the case in the ventral horn, any more than in other neuronal systems. On the other hand, because the micro-electrodes are known to record action currents in a highly localized way, the absence of significant electrical changes due to motoneurons in Fig. 3*b* does not constitute definitive evidence against this possibility; nor, for the same and additional reasons, does the absence of changes in the interneuronal discharge with change in the electrical field due to impulses in the motoneuronal somas (Fig. 6).

In any event, centrifugal as well as centripetal impulses in the motor axons would be expected to invade the recurrent collaterals, and Lorente de Nó (16, 17) has demonstrated that the electrical responses of motoneuronal somas to antidromic and to synaptic stimulation are essentially identical. It is, therefore, reasonable to extend the present findings to motoneurons stimulated synaptically. Thus, the internuncial system in the ventral horn may act as a significant correlating system, each interneuron being affected by the discharges of many motoneurons (and perhaps of other neurons), and being facilitated or inhibited according to the population of active motor cells. It does, to be sure, seem probable that the virtually simultaneous excitation of the majority of the motoneurons sending axons into any motor nerve would occur but rarely in normal life, and one would anticipate that physiologically evoked myotactic and other reflexes would initiate interneuronal responses more like those shown in records *b-e* of Fig. 3 than in records *f* and *g* of that figure.

One possible role of the interneurons is suggested by a formal similarity which exists between the temporal courses of (i) the frequency of the interneuronal action potentials appearing in the ventral horn as a sequel to an antidromic volley (cf. Figs. 1 and 2), and (ii) the degree to which the two-neuron arc discharge of a pool of motoneurons is inhibited by an antecedent antidromic volley in the axons of adjacent pools of motoneurons (cf. 20, Fig. 5). However, although it was in fact the discovery of (ii) which led to the successful search for the interneuronal activity described in this paper, it is not yet known whether the two phenomena are causally related. As in other instances, a decision must await upon as yet unavailable neuroanatomical knowledge.

The most striking feature of the interneuronal activity described above is that a single volley of centripetal motor impulses is capable of causing the individual interneurons to respond in such a regular pattern with so many impulses, and at frequencies as high as 1500 per sec. (cf. Figs. 2, 3, and 4). Even centripetal volleys involving but a small fraction of the motor axons of a peripheral nerve evoke repetitive unitary discharges recurring at intervals of 1 msec. or less (cf. Fig. 3*d* and *e*). Motoneurons (3, 4, 19) and some types of spinal interneurons (11, 12) ordinarily do not discharge at such high frequencies. On the other hand, there is evidence that sufficiently powerful synaptically-delivered stimuli can discharge these types of cells at very high frequencies. Lorente de Nó (15) has demonstrated that motoneurons can discharge successive impulses at an interval of 0.6 msec., thereby establishing an upper limit for the absolutely refractory period; and Lloyd (12, Fig. 7) has depicted an interneuron of the dorsal horn which responded to a single large dorsal root volley with a series of 8 action potentials appearing at a frequency of *ca.* 700 per sec. Moreover, there have been reported instances in which single cortical units, responding to stimuli presumably physiological in nature, have exhibited repetitive, high frequency action potentials, similar to those here described but normally involving somewhat lower frequencies (*i.e.*, 700–1000 per sec.) and a total of only 3–5 impulses (2, 6, 22). In the case of pyramidal units, Adrian and Moruzzi found more prolonged discharges of higher frequency (*i.e.*, up to 50 impulses at maximal rates of *ca.* 1500 per sec.) under abnormal conditions, as for example when strychnine or electrical stimuli were directly applied to the cortex. In the case of the spinal interneurons under discussion these abnormal conditions were not present. One cannot, however, rigorously exclude the possibility that the cells may have been rendered to some extent abnormal as a result of the insertion nearby of the recording micro-electrode. There is considerable evidence (see above) that the discharges do not depend on injury, but final proof that their character is entirely independent of it would require some such experiment as the recording of similar patterns of impulses in the axons of the cells at a distance from the somatic regions in the ventral gray matter where the impulses are initiated.

In summary, it would seem possible that certain types of interneurons

discharge in the pattern described above for interneurons of the ventral horn. In any event, it is of considerable interest to find in the spinal cord a type of neural unit which, in the absence of a convulsant drug or directly applied electrical stimuli, can respond to a single incoming volley of impulses with a regular sequence of action potentials at frequencies initially as high as 1500 per sec. (cf. Fig. 2).

### SUMMARY

Centripetal volleys of impulses which enter the spinal cord over alpha fibers of ventral (motor) roots in cats and rabbits evoke in the ipsilateral ventral horn action potentials (spikes) which persist, in progressively decreasing numbers, for 30–50 msec. The action potentials do not represent repetitive centripetal discharges from the periphery, and no comparable centrifugal impulses in motor axons have been detected. It is, therefore, inferred that they represent the activity of interneurons located in the ventral horn. The available evidence suggests that the discharges are not injury effects associated with the presence of the recording micro-electrode.

Impulses in many motor axons regulate the discharges. In general, as the size of an antidromic volley is increased, individual neurons respond with an increasing number of spikes at increasing frequencies and decreasing latencies. The first action potential has a *minimum* latency, measured from the time of arrival of the centripetal volley at the somas of the motoneurons, of 0.7 msec. The first two or three action potentials are sometimes spaced at intervals as short as 0.6–0.7 msec., i.e., the frequency is about 1500 per second. The succeeding impulses, which may total as many as fifteen, are spaced in a regular pattern at progressively increasing intervals.

A neuron's discharge to a centripetal volley in one deafferented motor nerve can be conditioned (augmented or decreased) by simultaneous or preceding volleys in a second deafferented motor nerve. The neurons frequently are not discharged by dorsal root volleys sufficing to activate relatively few motoneurons; in other instances the same neuron can be thrown into activity by either an antidromic motor volley or a dorsal root volley.

It is reasonable to extrapolate the present findings to instances in which motoneurons are synaptically rather than antidromically stimulated. Thus the internuncial system in the ventral horn may act as a significant correlating system.

Attention is directed to the regular pattern of discharge at surprisingly high initial frequencies, and it is suggested that some types of interneurons may normally exhibit this type of activity.

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# TONIC AND REFLEX FUNCTIONS OF MEDULLARY SYMPATHETIC CARDIOVASCULAR CENTERS

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IN 1870 it was demonstrated that the maintenance of normal cardiovascular function is dependent upon the integrity of structures which are located in the rostral portion of the medulla oblongata of the brain stem. (For studies of Dittmar and of Owsjannikow, see 2.) It was later suggested by Porter (18) that this bulbar system might be subdivided into a "vasoreflex" center and a "vasotonic" center. In an attempt to localize these bulbar centers with greater accuracy, Ranson and Billingsley (20) explored the floor of the fourth ventricle with stimulating electrodes searching for vasomotor reactions. They identified relatively discrete points which yield significant responses to low intensity stimulation:—a pressor point in the fovea inferior at the apex of the ala cinerea and a depressor point in the area postrema just lateral to the obex. They also found somewhat variable pressor responses in the region of the facial colliculus which they attributed to Porter's "vasotonic" center. Chen, Lim, Wang, and Yi confirmed the presence of the pressor and depressor points and contended that the pressor point represents a center concerned with the excitation of the entire sympathetic system (6) while the depressor point is concerned with the inhibition of the sympathetic system (10). On the other hand, the concept that these points on the floor of the fourth ventricle represent integrative centers for pressor and depressor functions has been challenged by Scott (21). He found that destruction of these regions by cautery did not interfere with normal pressor and depressor functions except in so far as there was damage to afferent vasomotor pathways.

These divergent points of view were resolved by exploring the deeper structures of the medulla, a procedure which was made possible by the introduction of the Horsley-Clarke stereotaxic instrument. Orienting fine needle electrodes with the aid of this instrument, Wang and Ranson (22) explored the entire substance of the brain stem from the pons to the decussation of the pyramids. A similar though less extensive exploration has been carried out by Monnier (12). This method revealed extensive pressor and depressor regions within the reticular formation of the medulla which yield maximal or near maximal responses to low intensity stimulation. This evidence served to confirm the contention of Scott that the discrete points on the floor of the fourth ventricle are of little functional significance, since they are merely points where the deeper lying centers come close enough to the surface to be stimulated by superficial electrodes.

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It seems pertinent to re-evaluate the functional significance of these pressor and depressor centers, considering them as reticular systems rather than as superficial regions on the floor of the fourth ventricle. To add greater clarity to the analysis, use has been made of the technique of recording directly from the peripheral nerves whose activity is controlled by these centers; a method which has been applied with notable success by Pitts and his associates in investigating the problem of hypothalamic influence on cardiovascular control (16) and the function of the bulbar respiratory centers (14). The scope of the present analysis is restricted to the sympathetic vasoconstrictor and cardioaccelerator system, since the enhancement or inhibition of the tonic activity in these effectors is the major factor responsible for the pressor and depressor responses obtained by central stimulation. This study does not concern itself with other cardiovascular regulators such as the vagal supply of the heart and the vasodilator efferents, even though it is probable that these latter systems are in close functional association with the constrictor-accelerator system.

#### METHODS

All experiments were performed on cats. In most instances they were lightly anesthetized with chloralose (40 mg./kg.); in one series decerebrate cats (intercollicular transection) were studied without the use of continuous anesthesia. Nerve potentials were detected with silver-silver chloride brush tipped electrodes, amplified with conventional capacity coupled amplifiers, and recorded by means of a General Electric mirror oscillograph projecting upon a bromide paper camera. In most experiments recordings from the inferior cardiac nerve were employed as a sensitive index to the activity in the sympathetic outflow to the cardiovascular system, since the cardioaccelerator fibres of this nerve exhibit a high degree of tonic activity (4). To gain access to the nerve the chest was opened and the animal maintained with artificial respiration. In some experiments recordings were obtained from fibres dissected from the cervical sympathetic trunk, in which case thoracotomy and artificial respiration were not required. Fibres were selected from this nerve which responded to stimulation of the ipsilateral pressor center and which exhibited reflex inhibition in their activity with a rise in blood pressure and which were therefore assumed to be functionally associated with the vasomotor system.

Stimulation of the brain stem was carried out with bipolar needle electrodes oriented by means of the Horsley-Clarke stereotaxic instrument. This instrument was equipped with two electrode carriers making it possible to stimulate two points in the brain simultaneously. Stimuli were obtained from a pair of independent stimulators which gave brief condenser discharges (time constant: 0.1 msec.) of any desired frequency and intensity. In a preliminary series of exploratory experiments, the posterior region of the skull was removed and the exploring electrode passed through the cerebellum into the medulla. Respiration was recorded simultaneously with blood pressure and so far as possible complications in the blood pressure records produced by the coincidental stimulation of the respiratory centers were dissociated from true vasomotor responses. At the conclusion of an experiment the brain was exsanguinated and perfused with formalin *in situ*, removed and sectioned by the freezing method of Marshall (11), and stained with the routine Weil procedure. The positions of the stimulating electrodes were accurately identified on these stained sections. In experiments involving transection of the brain stem, the floor of the fourth ventricle was visualized by removing the cerebellum. A lapse of at least one hour intervened after the decerebellation, and the ability of the animal to maintain normal blood pressure and normal respiration during this interval was taken as an index to absence of injury to the structures within the substance of the medulla. Transections were performed with the single sweep of a sharp scalpel. In order to avoid cutting into the basilar artery, care was taken to avoid bringing the blade of the scalpel all the way to the floor of the brain case in the mid-line. This usually resulted in an incomplete transection of the pyram-

idal tracts, but for the purpose of these experiments a section which transected the entire reticular substance was considered "complete." After an experiment the brains were hardened in formalin and examined under binocular magnification to ascertain the exact level and completeness of the transection.

## RESULTS

*Localization of centers.* In order to become familiar with the localization of the bulbar cardiovascular centers, a preliminary series of exploratory experiments were carried out which were essentially a repetition of those reported by Wang and Ranson (20). A total of 28 cats was studied and blood pressure recordings were obtained of the responses to stimulation of an average of 100 different points in the brain stem of each animal. In contrast to the method of Wang and Ranson where repetitive stimuli were obtained from a conventional inductorium, brief condensor discharges were employed at a frequency of 200/sec. and a peak intensity measured with the electrodes in situ of 8 volts. This form of stimulation was selected because it is in the range of optimal frequency (8) and restricts the area directly stimulated to a volume of brain tissue at the electrode tips of about 1 mm.<sup>3</sup> (13). Careful analyses of the responses obtained were correlated with direct anatomical identification of the electrode positions and maps of responsive areas constructed. In general these maps were in good agreement with those published by Wang and Ranson. There were some minor discrepancies in the exact delimitation of the centers and there was considerable divergence in the distribution of submaximal responses. These discrepancies might well be related to differences in technique, notably in the different form of stimuli employed. Until some basis is presented for identifying the discrete anatomical elements which constitute the centers, these discrepancies would seem to be of little significance—especially when one bears in mind that even with uniform technique successive animals may show considerable variation in the precise distribution of responsive points. This exploratory study therefore does not warrant publication in detail, but is summarized briefly in the following paragraphs and in Figure 1.<sup>2</sup>

At the level of the pons, pressor responses are sparsely scattered and usually of small magnitude. Just caudal to the pons in the region overlying the trapezoid body, maximal pressor responses amounting to 100 mm. or more are obtained throughout a large area of the lateral reticular formation ventral to the nuclear gray and dorsal to the superior olivary nucleus. Although maximal responses are found most uniformly in this lateral region, the area of pressor response extends as far medial as the mid-line where responses of 80 to 100 mm. are frequently encountered. This region of maximal

<sup>2</sup> It should be emphasized that the diagrams in Figure 1 are based upon composite maps which to some extent misrepresent the pattern of responses which might be obtained in any specific animal. This is especially true of the implied massive continuity of the centers. In any given animal certain local areas may fail to show a response even though the composite picture obtained from many animals indicates that these areas are usually responsive.



pressor response continues down through the region of the facial nucleus, but near the caudal pole of the facial nucleus pressor responses disappear from the mid-line and become progressively more restricted to the lateral wings of the reticular formation. These lateral extensions of the pressor re-

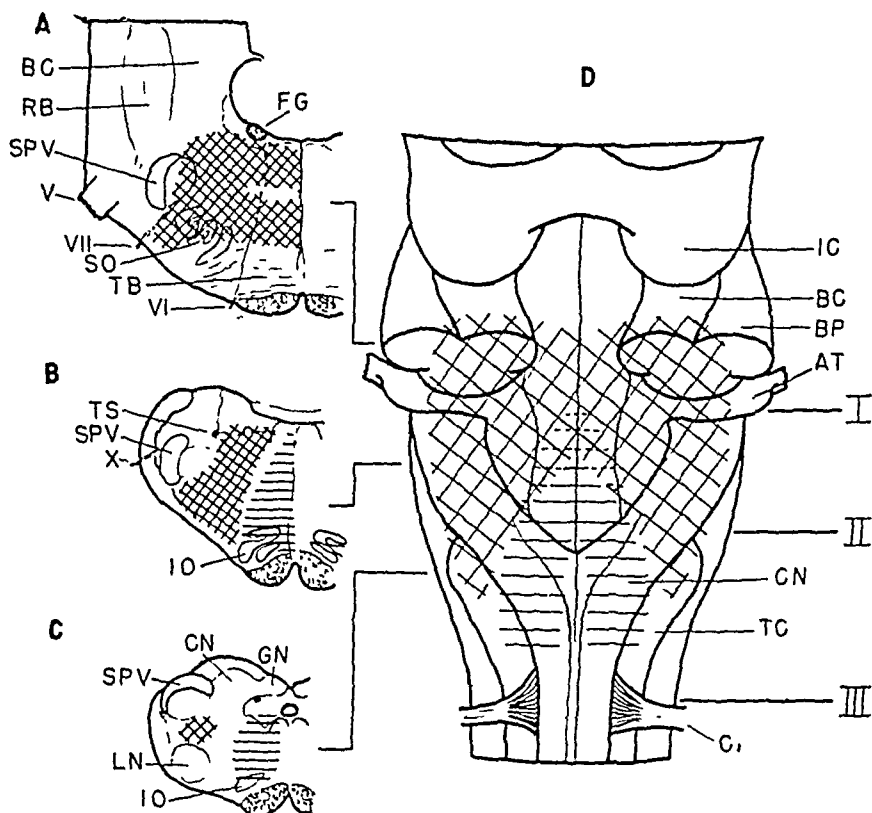


FIG. 1. Localization of pressor and depressor centers in the brain stem of the cat. Pressor regions indicated by cross hatching; depressor regions by horizontal ruling. A-C:—cross sections through medulla at levels indicated by guide lines to D; D:—semi-diagrammatic projection of pressor and depressor regions onto the dorsal surface of the brain stem viewed with the cerebellar peduncles cut across and the cerebellum removed.

Legend:—AT: auditory tubercle; BC: brachium conjunctiva; BP: brachium pontis; C<sub>1</sub>: first cervical nerve; CN: cuneate nucleus; FG: facial genu; GN: gracile nucleus; IC: inferior colliculus; IO: inferior olivary nucleus; LN: lateral reticular nucleus; RB: rests-form body; SO: superior olivary nucleus; SPV: spinal trigeminal tract; TB: trapezoid body; TC: tuberculum cinereum; TS: tractus solitarius; V, VI, VII, X: corresponding cranial nerves; I, II, III: levels of transection discussed in text.

gion continue caudally dorso-lateral to the inferior olivary nucleus. At the level of the decussation of the medial lemniscus, scattered points yielding moderate pressor responses become somewhat more numerous especially throughout the dorsal columns, but these submaximal responses appear to be due to stimulation of the ascending afferent pathways in this region.

Typical maximal pressor responses are restricted to a relatively small area overlying the lateral reticular nucleus.

Moderate depressor responses are found at points scattered through the tegmental structures of the pons overlying the medial lemniscus. Just caudal to the pons, however, depressor responses almost entirely disappear except for the region of the vestibular nuclei and a fairly discrete area near the mid-line. These mid-line depressor points appear to constitute a band of tissue represented in Figure 1A by the gap in the pressor region and are suggestive of descending depressor pathways, possibly including the fibres in this region which descend from the hypothalamus (3) but which do not mediate hypothalamic pressor responses (23). Significant depressor regions are not encountered until one passes caudal to the facial nucleus. As the rostral pole of the inferior olivary nucleus is approached, maximal depressor responses appear in the mid-ventral region of the reticular formation overlying the pyramidal tracts. This depressor region becomes progressively larger, extending dorsally to the nuclear gray and somewhat laterally so as to include most of the medial reticular formation throughout the length of the inferior olive. Maximal depressor responses continue to be found in the medial reticular formation down through the decussation of the medial lemniscus.

*Origin of tonic activity.* Inspection of Figure 1D reveals that the centers are so located as to make possible differential elimination of pressor and depressor regions by transecting the brain stem at appropriate levels. Transection through the auditory tubercle (Fig. 1D-I) removes a significant portion of the rostral pressor region without encroaching on the depressor region. Section at a level slightly rostral to the obex (Fig. 1D-II) removes a large part of the pressor region while still leaving a major portion of the depressor region intact. Finally, section of the cord at C<sub>1</sub> (Fig. 1D-III) yields a spinal animal isolated from the bulbar pressor and depressor centers. In a series of 9 cats lightly anesthetized with chloralose and an additional series of 5 unanesthetized decerebrate cats, serial transections of the brain stem have been carried out and the resulting deficiencies studied. The results obtained from the two series were quite comparable.

Transections as far caudally as the lower third of the pons have no significant effect on blood pressure or on the tonic activity recorded in the inferior cardiac nerve. Transection at lower levels yield results such as those illustrated in Figure 2. The initial record in this figure illustrates the characteristic tone in the inferior cardiac nerve of a normal preparation; the mean blood pressure of 108 mm. is typical for cats with open thorax. Subsequent recordings show the changes produced by transecting the brain stem at the levels indicated. The amount of amplification of the nerve potentials relative to the control level is given for each segment of the recording. It will be noted that section at I leads to a considerable fall in mean blood pressure correlated with a significant reduction in tonic accelerator activity. Section at II produces a maximal fall in blood pressure together with a

complete disappearance of activity in the inferior cardiac nerve. This state of atonia was rather striking in comparison with the condition seen in the spinal animal (1), where experience has shown that there is never as complete an absence of tone as that seen here in the low bulbar animal. The transformation from this atonia of the low bulbar animal to the typical hypotonia of the spinal animal by section at III is shown in the latter recordings of Figure 2.

Repeated experiments of this type, including transections at levels intermediate to those illustrated, made it clear that loss of tonic activity correlates closely with a loss of corresponding portions of the pressor region of

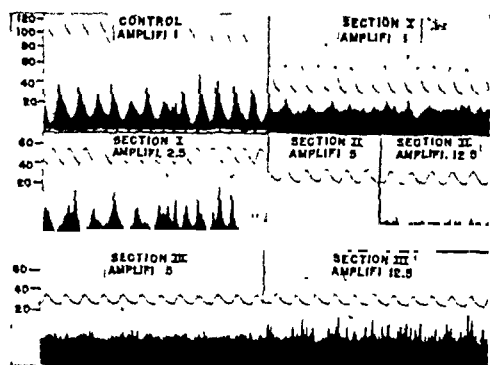


FIG. 2. Blood pressure and tonic activity in the inferior cardiac nerve of an unanesthetized decerebrate and decerebellate cat. Sections indicated refer to transections of the brain stem at the levels shown in Figure 1D. Amount of amplification of nerve potentials indicated relative to control level. Scales at left give blood pressure in mm. Hg. Time signal in all recordings gives 1/5th second intervals.

the brain as outlined by exploratory stimulation. In addition, it is evident that under the conditions of these experiments the depressor region exhibited tonic activity. The uniform appearance of low grade cardioaccelerator tone when the cervical cord of the atonic low bulbar animal is sectioned must indicate a release from tonic depressor activity descending to the spinal cardiovascular centers from the depressor center in the medulla.

*Site of reflex mediation.* Cardiovascular reflexes were tested in the course of the transection experiments described above. Single shocks of moderately strong intensity delivered to the central end of the sciatic nerve produce a volley of impulses in the inferior cardiac nerve. When given repetitively, these stimuli elicit the typical sciatic pressor reflex. In Figure 3A and B are illustrated the results obtained from two reasonably typical experiments in which the effect of transection on the single shock response was studied. In the first animal (A) good tonic activity was present before the transection and single shocks to the sciatic produced a heavy barrage of impulses in the inferior cardiac nerve. Subsequent section at I greatly depressed this response. The recording shown here is typical of these experiments in the variability of the response that is obtained after section at this level. It will be noted that there is a definite response following the first stimulus, no response following the last, and the intermediate responses are question-

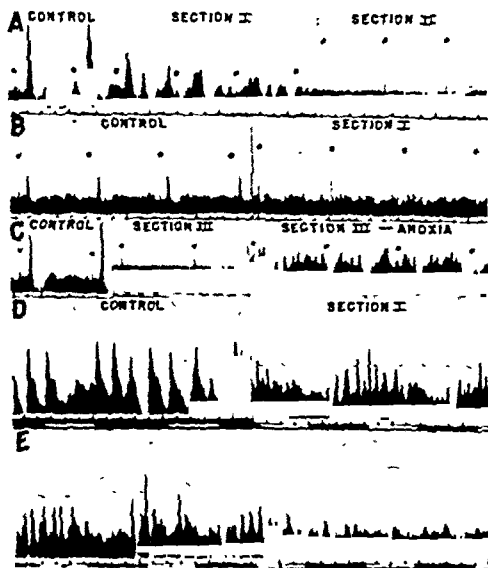
able. Transection of the brain stem at II completely abolished both the tonus and the reflex response. The second series were obtained from an animal which exhibited a relatively low initial tone. In this case (3B) the reflex response was completely abolished by section at I.

It appeared possible that this state of areflexia following loss of the pressor center might merely be due to a loss of the excitatory tone descending from the medulla to the spinal centers. If this were true, some alternate procedure for increasing the excitability of the spinal centers might permit

FIG. 3. Influence of brain stem transections (cf. Fig. 1D) on cardiovascular reflexes as recorded in the inferior cardiac nerve.

A-C:—Single shocks to sciatic nerve; stimulus artefacts indicated by black dots. A:—amplification in right hand recording 5 times that in control; B:—amplification in right hand recording 2.5 times that in control; C:—ventilation with nitrogen to produce anoxic stimulation of spinal centers fails to restore reflex responses, all recordings at same amplification.

D-E:—Decerebrate preparation showing accentuation of pulmonary depressor reflex following removal of rostral pressor region (D) and inhibition of cardio-accelerator tone with vagal slowing of the heart resulting from a rise in blood pressure produced by adrenalin (E). White signal beneath time marker indicates inspiration.



the sciatic pressor reflex to become patent as a purely spinal reflex (5). Recent evidence (1) has demonstrated that anoxia has a marked stimulatory action on spinal cardiovascular centers. Therefore low bulbar and spinal animals were subjected to anoxia while the response to strong stimulation of the sciatic nerve was being tested. In spite of repeated attempts to elicit this reflex employing every conceivable variation in method, it has not been possible to demonstrate a pressor reflex in the acute preparation once the pressor region in the medulla has been eliminated. A typical anoxia experiment with single shock stimulation is shown in Figure 3C; repetitive stimulation at either low or high frequency was equally ineffective in spite of the appreciable increase in tonic activity produced by the anoxia. In the light of this evidence, it seems justified to regard the somatic pressor reflex as undergoing true reflex mediation through the bulbar pressor center, thus reinforcing any component of this reflex which may be purely spinal in nature.

In contrast to pressor reflexes which show great impairment after section at I, depressor reflexes remain quite active. In fact the pulmonary reflex

which acts during inspiration to depress cardioaccelerator tone (4) is frequently more pronounced after eliminating the rostral portion of the pressor center (Fig. 3D). Furthermore, a rise in blood pressure produced by injecting adrenalin gives a reasonably normal depressor reflex after section at I as seen in Figure 3E. Note that the rise in blood pressure results in vagal slowing of the heart as well as sympathetic inhibition. This indicates that depressor reflexes remain patent as long as the depressor center and its afferent inflow are intact.

*Bilateral organization of centers.* In general the sympathetic outflow to the cardiovascular system is not concerned with the isolated activity of discrete units but exhibits changes in the functional activity of the system as a whole. As a consequence one might expect close interrelation and extensive decussation of the bilateral halves of the bulbar system. For a direct analysis of this relationship, the inferior cardiac nerve was prepared for recording cardioaccelerator activity and from the cervical sympathetic trunk of the same side groups of fibres were isolated which gave evidence of being vasomotor in function. Two pairs of electrodes were then placed at bilaterally symmetrical positions in the pressor centers of each side of the medulla. It was thus possible to record alternately from either cardioaccelerator or vasomotor fibres while either the ipsilateral or contralateral pressor center was being stimulated. As an experimental expedient, cardioaccelerator responses were usually tested with single shocks to the pressor center, while repetitive stimulation was required to obtain significant responses in the cervical sympathetic. Supplementary experiments were performed which gave evidence that the form of stimulation was not responsible for any qualitative difference in the responses obtained.

The responses obtained in the inferior cardiac nerve of one side from sending identical single shock stimuli to the ipsilateral and contralateral pressor centers are shown in Figure 4A and B. It will be seen that large responses were obtained from both sides of the medulla. Experiments of this type showed a fairly uniform tendency for the ipsilateral response to be on the average somewhat greater than the contralateral response, indicating that there is not complete decussation of the control of cardioaccelerator activity. Nevertheless, a large degree of decussation is apparent. This relationship is to be contrasted with the vasomotor responses obtained from the cervical sympathetic of the same preparation illustrated in Figure 4C and D. Ipsilateral stimulation (C) produced a large amount of activity in the nerve; yet there was barely a suggestion of activity produced by stimulation of the contralateral pressor center (D). In spite of this absence of contralateral response, the rise in blood pressure (just beginning at the end of the portion of the record shown) was from 100 mm. to 215 mm. in the course of ten seconds of contralateral stimulation. This absence of a contralateral response was quite typical, although in some preparations a very brief burst of activity may appear at the moment of stimulation. With extensive exploration of the medulla pressor points can be found which give significant

activity in the contralateral nerve, but the scattered distribution of these points would appear to identify these responses as being the result of chance stimulation of afferent pathways to the contralateral side which are not an integral part of the centers as a whole. Studies on more than 20 animals have

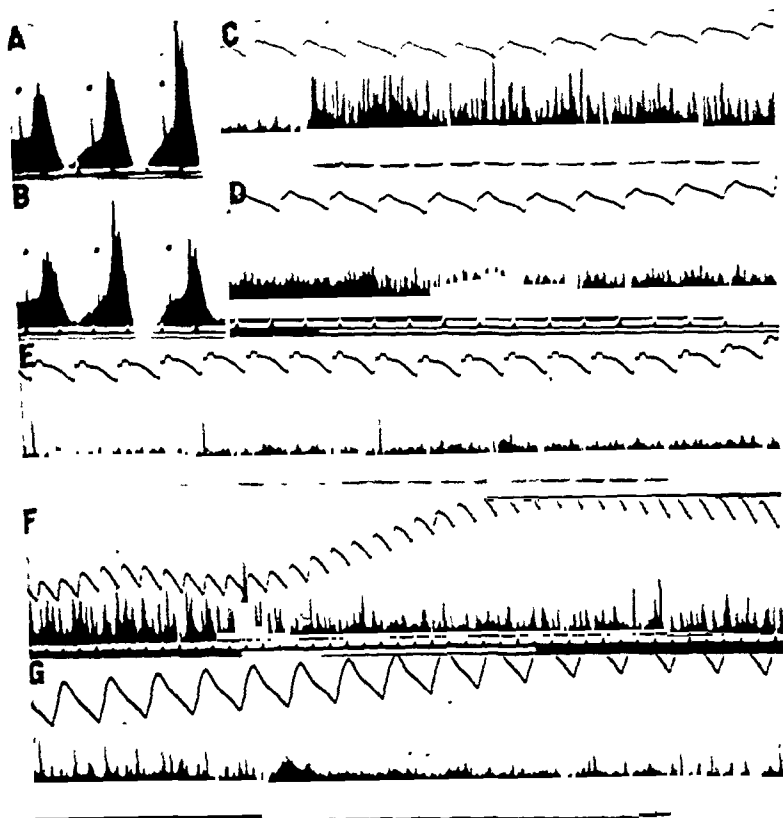


FIG. 4. Effects of direct stimulation of bulbar pressor centers on peripheral nerve response. Single shock artefacts indicated by black dots; repetitive stimulation indicated by white signal beneath time marker. A-D from same preparation. A:—Response in inferior cardiac nerve to stimulating ipsilateral pressor center; B:—Identical stimulation of contralateral pressor center; C:—Response in cervical sympathetic to repetitive stimulation of ipsilateral pressor center; D:—Identical stimulation of contralateral pressor center.

E-G:—Examples of inhibition of vasomotor activity in the cervical sympathetic by repetitive stimulation (signal) of the contralateral pressor center. E:—single fibre tonically active; F:—exaggerated tonic activity in shock; G:—fibres activated by repetitive stimulation of hypothalamic centers throughout recording.

revealed that as a rule there is no significant response in the cervical sympathetic to stimulation of the contralateral pressor center.

Of even greater interest than the lack of contralateral excitation was the presence of contralateral inhibition with stimulation of the pressor center on one side. Although there is relatively little tonic activity present in the

typical cervical sympathetic preparation, dissection will usually reveal scattered fibres which exhibit tonic activity of low frequency. Such a fibre, which fired quite regularly at about once a second, is shown in Figure 4E. Stimulation of the contralateral pressor center inhibited the rhythmic firing of this fibre. In another instance an animal was encountered which exhibited an unusually high degree of tonic activity in the cervical sympathetic correlated with the development of circulatory shock. The partial inhibition of this tone by contralateral pressor stimulation is shown in Figure 4F. In this instance the recording speed was slow enough so that the pressor response to the contralateral stimulation may be seen in the figure. A more controllable procedure for producing activity in the cervical sympathetic is to subject the hypothalamic pressor area to repetitive stimulation. This results in a fairly steady state of activity in small groups of fibres dissected from the nerve. When the contralateral pressor center is stimulated simultaneously with the ipsilateral hypothalamic pressor center, there is a marked inhibition of the activity as shown in Figure 4G. As yet exhaustive exploratory experiments have not been carried out to determine whether this contralateral inhibition is typical of the entire bulbar pressor region, but it is not to be regarded as a chance occurrence since it may be obtained quite uniformly from the lateral regions of the reticular formation which are most uniform in yielding maximal pressor responses.

### DISCUSSION

The use of the Horsley-Clarke stereotaxic instrument to locate "centers" covering the extensive regions represented by the bulbar cardiovascular centers is open to some justifiable criticism owing to the fact that it is impossible to determine whether the electrodes are stimulating afferent, association, or efferent elements. This criticism is only partially met by the assumption that a response of large magnitude must represent a fairly generalized excitation of the pool of closely associated neurones constituting the center. In the present study, however, the transection experiments serve to confirm the exploratory experiments. The fact that functional deficits resulting from transections at various levels are in close agreement with what would be anticipated from the exploratory experiments leaves little doubt that the centers as identified by exploratory stimulation have a real functional significance.

The observations on tonic activity would indicate that as a first approximation the bulbar pressor center may be regarded as an extensive pool of neuronal elements each of which is contributing its fractional part to the general excitatory state and tonic function of the sympathetic outflow to the cardiovascular system. In respect to the depressor center, the demonstration of tonic depressor activity is of particular interest. In view of the nature of these experiments, any conclusions as to the degree of depressor tone in the normal animal are unwarranted. The real importance of this finding, however, is that it establishes the depressor center as a functional entity rather

than merely a region through which inhibitory afferents travel to reach the pressor center, as has sometimes been proposed (21). The records demonstrate that in addition to whatever intrabulbar association pathways there may be, there must also be pathways descending from the caudal portion of the medulla to the spinal cardiovascular centers which are capable of tonically inhibiting their activity. This tonic inhibitory activity originates at a level that corresponds with the depressor center as identified by exploratory stimulation. The descending pathway from this depressor center doubtlessly involves the tracts in the dorsolateral columns of the cord which Lim, Wang, and Yi (10) found to mediate the depressor response obtained by stimulating the area postrema.

This study has failed to reveal any evidence of a vasotonic center anatomically distinct from a vasoreflex center, since the transection experiments revealed a very close correlation between tonic and reflex deficits after removal of portions of the bulbar centers. The only basis for such a concept appears to be the experiments reported by Porter (17, 18, 19) which he recognized as being highly indirect and inconclusive. In the absence of any other evidence, therefore, the most acceptable view would be to regard the central mechanisms for both tonic and reflex functions as being dependent upon excitability changes in the same cellular elements, whether these changes in excitability be due to persistent environmental influences or to specific afferent bombardment.

It has been demonstrated that there is extensive decussation in the bulbar control of cardioaccelerator activity, while there is no significant decussation in the excitatory influences exerted by the bulbar centers on the cervical sympathetic. In addition it has been possible to demonstrate reciprocal inhibition of the cervical sympathetic on one side with stimulation of the contralateral bulbar pressor center. Since these cervical sympathetic fibres gave evidence of being associated with vasomotor control, this pattern of organization makes possible a selective control over the blood supply to structures on one side of the head. These observations raise the question as to whether such a unilateral organization might be found more generally in the control of the sympathetic outflow to the vasomotor system. Such a viewpoint would be substantiated by the fact that the pressor response to stimulation of the bulbar pressor center of one side is abolished by ipsilateral hemisection of the cervical cord (7, 9), although there is some evidence of vasomotor decussation at lower spinal levels (9). Even in the cervical sympathetic, however, the dissociation of the bilateral halves of the pressor system does not extend throughout the entire neuraxis, since the hypothalamic centers exhibit extensive decussation in their influence on the activity in the cervical sympathetic (15). More extensive investigations of the central control of activity in the various levels of vasomotor outflow must be carried out before it will be possible to assess the type of bilateral integration that takes place in the vasomotor system as a whole.

This investigation was undertaken at the suggestion of Dr. Robert F. Pitts who



generously placed his apparatus at the author's disposal and to whom the author is deeply indebted both for training in the technics and for many valuable suggestions and constructive criticisms throughout the course of this study.

### SUMMARY

1. In confirmation of previous studies, pressor and depressor regions in the medulla of the cat have been identified by exploratory stimulation with the aid of the Horsley-Clarke stereotaxic instrument. The pressor center was found to occupy an extensive region of the lateral reticular formation in the rostral two-thirds of the medulla, while the depressor center includes a greater part of the medial reticular formation in the caudal half of the medulla.

2. The functional significance of the pressor center is confirmed by the fact that transections designed to remove a portion of the pressor region produce an equivalent reduction in blood pressure and cardioaccelerator tone, the latter having been observed directly by recording the activity in the inferior cardiac nerve.

3. The depressor center is shown to be functionally significant in that it is capable of tonically inhibiting the activity of the spinal cardiovascular centers.

4. Somatic pressor reflexes produced by stimulating the sciatic nerve are dependent upon the integrity of the bulbar pressor center.

5. Depressor reflexes remain functional as long as the depressor center in the medulla is intact.

6. Recordings from the peripheral nerves demonstrate that stimulation of the bulbar pressor center of one side produces increased activity in the inferior cardiac nerves bilaterally, while in the cervical sympathetic the excitatory influence of the bulbar pressor center of one side is restricted to the ipsilateral nerve with a reciprocal inhibition of activity in the contralateral nerve. In the case of the cervical sympathetic this indicates the possibility of selective control over the activity in the sympathetic outflow to structures on one side of the head.

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# BRAIN STEM FACILITATION OF CORTICAL MOTOR RESPONSE

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RECENT interest in disorders of motion of an extrapyramidal type has placed particular emphasis upon hyperkinesias or other states of abnormal activity which, in general, appear to be release phenomena resulting from the impairment of inhibitory neural mechanisms. These, however, are not the only manifestations of extrapyramidal disorder and the hypokinesia and slowness of movement of Parkinson's disease might, for example, be due to the impairment of neural mechanisms which normally contribute a background of excitement and so facilitate motion. In an experimental study of brain stem mechanisms influencing motor activity, begun in 1944 (12), stimulation of the bulbar reticular formation was found both to inhibit and to facilitate movements induced either reflexly or by stimulating the motor cortex. While the inhibitory influence was subsequently found to be intrinsically bulbar (14), further study indicated that the facilitation encountered resulted from stimulating a system of connections descending to the bulbar area from more rostral brain stem levels.

During the progress of this work, Murphy and Gellhorn (17) independently observed that stimulation of the hypothalamus facilitated cortically induced movement and include this result as part of the evidence which they present for a general hypothalamic activation of the cerebral cortex. The present paper, which summarizes our findings, is confirmatory of Murphy and Gellhorn's observation with respect to the hypothalamic facilitation of cortically induced movement, but provides an alternative interpretation of it. Our results indicate the existence of a system of facilitatory connections, arising possibly in the pallidum and certainly in the basal diencephalon, which pass down through the lower brain stem to enter the spinal cord. The facilitation of cortically induced movement obtained by stimulation of the hypothalamic part of this system would appear from the present study to occur, not within the cerebral cortex, but at spinal levels, and it can, in fact, be demonstrated with pyramidal tract responses after cortical extirpation.

## METHODS

In cats and monkeys under light chloroform anesthesia,<sup>3</sup> the exposed motor cortex was briefly stimulated with induction shocks at 2 sec. intervals, and the excursions of the opposite legs were recorded on a kymograph. In some instances the patellar reflex was repeatedly elicited with a solenoid similar to that employed by Johnson (7). Using the Hors-

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

<sup>2</sup> Grateful appreciation is expressed to J. M. Weaver for his assistance.

<sup>3</sup> The monkeys received a small amount of nembutal in addition.

ley-Clarke technique, the brain stem was stimulated in an exploratory fashion with 60 cycle sine wave current,<sup>4</sup> at intensities which did not by themselves evoke somatic motor activity. In two cats, hypothalamic points facilitating cortically induced movement were tested in the same experiment against motor activity elicited by stimulating the bulbar pyramid with an implanted electrode. In two other cats and one monkey, hypothalamic stimulation was similarly tested against pyramidal tract responses, but the cortex was extirpated by suction at the start of the experiment. In one cat, the medial portion of the thalamus was aspirated through an incision in the corpus callosum. In every instance, the sites of brain stem stimulation were subsequently determined from serial Weil-stained sections through the region explored.

## RESULTS

*Brain stem facilitation of motor activity.* The records shown in Figure 1A and C from the monkey and Figure 1B and D from the cat, illustrate

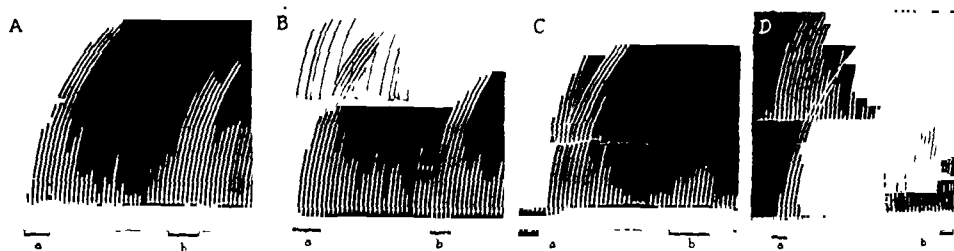


FIG. 1. Kymograph records of cortical motor responses (a) and patellar reflexes (b), evoked at 2 sec. intervals, facilitated by brain stem stimulation (signal) at diencephalic (A), midbrain (B), pontile (C) and bulbar (D) levels. The lower tracings in each instance are of the hindleg, the upper tracings in B-D, a, are of the foreleg. Records A and C are from the monkey, B and D are from the cat. Except in D, stimulation of the same brain stem point was tested against both cortically and reflexly induced movement.

the facilitation of cortically induced movement (a in each record) and of the patellar reflex (b in each record) which may be induced by stimulating the hypothalamus in A, the midbrain in B, the pons in C and the medulla oblongata in D. The augmented excursion of the cortical motor response is evident in each case; it is particularly striking in those instances in which the cortical stimulus was by itself subliminal. In the case of cortically induced movement, a gradually waning facilitatory after-discharge, lasting 30 sec. or more, often followed the cessation of brain stem stimulation. Though less frequent, this was sometimes also seen in records of the patellar reflex (Fig. 1A, b). These representative records make it clear that both cortically and reflexly induced movement can be facilitated, not only by exciting the hypothalamus, but as well by activating each lower brain stem level.

*Distribution of facilitatory sites in the monkey brain stem.* Upon a mid-sagittal reconstruction of the monkey's brain stem, shown in Figure 2, have been projected the sites whose stimulation facilitated cortically in-

<sup>4</sup> Frequencies varying from 5 to 300 per sec. were tested on facilitatory points with a Goodwin stimulator. Optimum effects were obtained with frequencies of 40 to 60 per sec.

duced movement. They are seen to be distributed both in the thalamus and more ventral parts of the diencephalon and to continue backward to the lower end of the brain stem in the central gray and tegmentum of the mid-brain, in the pontile tegmentum and in the bulbar reticular formation. A

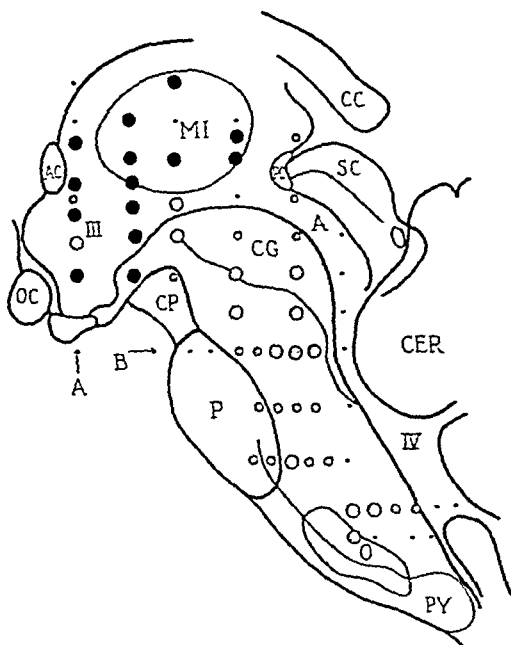


FIG. 2. Midsagittal reconstruction of the monkey's brain stem upon which are projected the sites whose stimulation facilitated cortically induced movements (dots and small and large stippled circles) or movements evoked from the bulbar pyramid after bilateral extirpation of the sensorimotor cortical area (solid black circles). In Figs. 2-4 the size of the symbols indicates the degree of facilitation, and abbreviations are as follows: A—aqueduct, AC—anterior commissure, AL—ansa lenticularis, BC—brachium conjunctivum, BIC—brachium of inferior colliculus, BP—brachium pontis, C—nucleus centralis, CC—corpus callosum, CER—cerebellum, CG—central gray, CL—nucleus centralis lateralis, CN—caudate nucleus, CP—cerebral peduncle, F—fornix, GP—globus pallidus, IC—internal capsule, L—lateral nucleus of thalamus, LST—lateral spinothalamic tract, M—medial nucleus of thalamus, MB—mammillary body, MI—massa intermedia, ML—medial lemniscus, MLF—medial longitudinal fasciculus, MT—mammillothalamic tract, O—inferior olive, OC—optic chiasma, OT—optic tract, P—pons, PC—posterior commissure, PU—putamen, PY—pyramid, RN—red nucleus, SC—superior colliculus, SN—subthalamic nucleus, VL—nucleus ventralis lateralis, VPL—nucleus ventralis posterolateralis, VPM—nucleus ventralis posteromedialis, III or 3V—third ventricle, IV—fourth ventricle.

cross section through level A of Figure 2, shown in Figure 3A, reveals that cortical motor responses on one side of the body were facilitated by stimulating both sides of the hypothalamus. Facilitatory effects obtained from stimulating in and adjacent to the ansa lenticularis (Fig. 3A) suggest that diencephalic components facilitating cortically induced movement may receive contributions from the globus pallidus.

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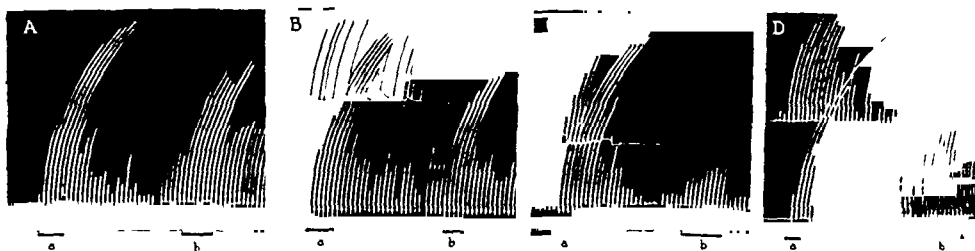


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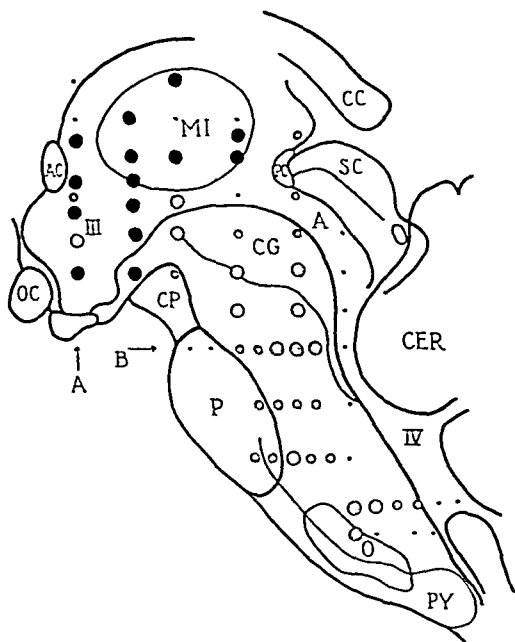


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In a cross section through the pons (level B in Fig. 2), shown in Figure 3B, are indicated the sites whose stimulation facilitated cortically induced movements of the right limbs. Facilitation is seen to have been obtained from both the ipsi- and contralateral pontile tegmentum, and the responsive sites appear to be distributed in three rather separate collections.

*Distribution of facilitatory sites in the cat brain stem.* Projection of facilitatory sites upon a reconstruction of the midsagittal plane of the cat's brain stem (Fig. 4A) reveals a distribution similar to that in the monkey, but with a somewhat greater representation of thalamic points facilitating cortical motor responses. These thalamic points were distributed chiefly in the

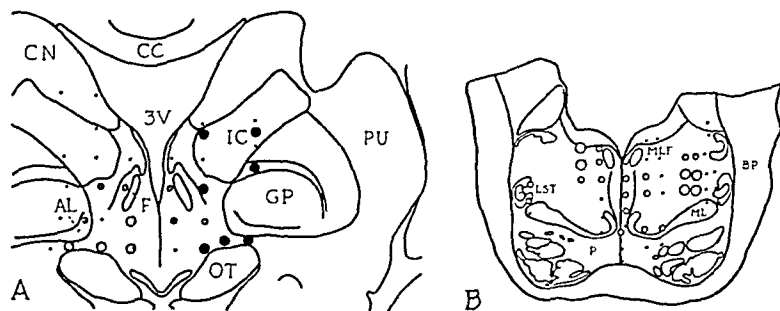


FIG. 3. Transverse sections through the anterior diencephalon (A) and pons (B) of the monkey, upon which are indicated the sites whose stimulation facilitated cortically induced movements (dots and small and large stippled circles) and movements evoked from the bulbar pyramid after extirpation of the sensorimotor cortex (solid black circles on the right half of A, which was the only portion of these two levels so tested). All movements were elicited from the left motor cortex or the left pyramid. The planes of these sections are indicated by arrows in Fig. 2.

midline and intralaminar group of nuclei and in the nucleus ventralis lateralis<sup>6</sup> (Fig. 4B and C). In the more ventral portion of the diencephalon, facilitation of cortically induced movement was readily obtained from both subthalamic and hypothalamic stimulation (Fig. 4A-C). From the caudal end of the diencephalon, an uninterrupted continuity of facilitatory sites passed backward into the midbrain (Fig. 4A), and at the mesencephalic level shown in Figure 4D, cortical motor responses were augmented by activating an area distributed rather widely in the periaqueductal gray and the dorsal midbrain tegmentum.

Difficulties attendant upon circumventing the bony tentorium have so far prevented study of the pontile portion of the cat's brain stem. In the medulla oblongata, where the influence of this brain stem facilitatory system was first encountered (12), increase of cortical motor response was obtained from stimulating the same bulbar reticular area, around the periphery of the

<sup>6</sup> Thalamic nuclei are differentiated after Magoun and McKinley (13) in the cat.



the patellar reflex, as well, lends support to this interpretation.<sup>6</sup> Murphy and Gellhorn (17) have suggested, on the other hand, that hypothalamic facilitation of cortically induced movement occurs within the cortex itself, by way of a recurrent system of hypothalamico-thalamo-cortical connections (18).

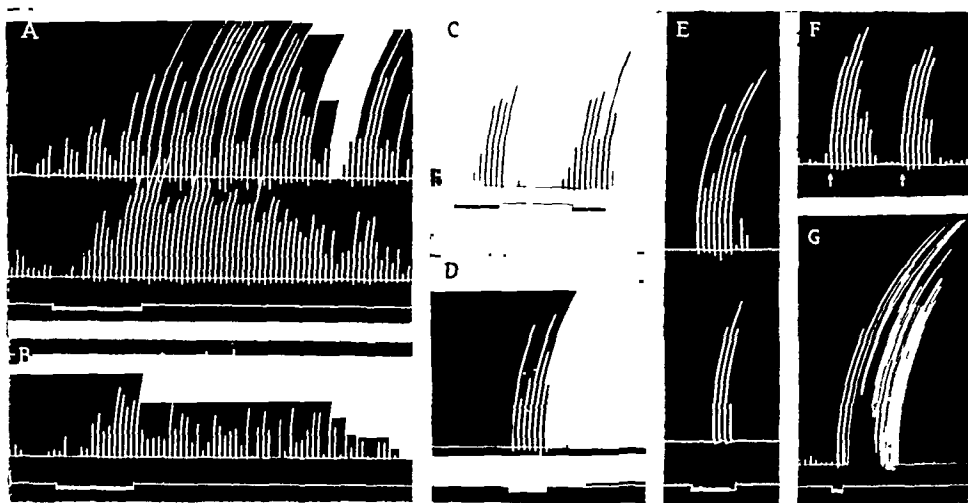


FIG. 5A and B. Facilitatory effect of diencephalic stimulation (signal) upon responses evoked from the motor cortex (A) and from the bulbar pyramid (B) in the cat. Both foreleg (upper tracing) and hindleg (lower tracing) movements were evoked from the cortex (A); only hindleg movements were obtained from the pyramid (B).

C. Facilitatory effect of diencephalic stimulation (signal) upon hindleg responses evoked from the motor cortex (left tracing) and bulbar pyramid (right tracing) in the cat.

D. Subliminal stimulation of the bulbar pyramid at 2 sec. intervals after bilateral cortical extirpation in the cat. A hindleg response appears during diencephalic stimulation (signal).

E. Subliminal stimulation of the bulbar pyramid at 2 sec. intervals after bilateral extirpation of the sensorimotor cortex in the monkey. Both foreleg (upper tracing) and hindleg (lower tracing) responses appear during diencephalic stimulation (signal).

F. Facilitation of hindleg responses from the monkey's motor cortex by pinching the toes (arrows).

G. Facilitation of hindleg responses from the monkey's motor cortex by stimulating the gracile nucleus (signal), followed by a convulsion.

Evidence bearing on this possibility has been obtained by comparing the effect of hypothalamic stimulation upon movements evoked from the motor cortex itself and movements elicited from fibers descending from the motor cortex in the bulbar pyramid. In the instance shown in Figure 5A and B, consecutive stimulation of the same hypothalamic point with the same intensity of current was effective in facilitating responses from the motor

<sup>6</sup> Facilitation of the patellar reflex may under certain conditions, however, be obtained by cortical activation (Dusser de Barenne and McCulloch, '33).

cortex (Fig. 5A) and from the pyramidal tract (Fig. 5B). It may also be noted that the facilitatory after-discharge, which often follows brain stem stimulation, was as evident with pyramidal tract responses (Fig. 5B) as when the motor cortex was stimulated (Fig. 5A). Another example in which hypothalamic stimulation facilitated both the response from the motor cortex and that from the pyramidal tract is shown in Figure 5C. Diencephalic points whose stimulation facilitated both motor cortical and pyramidal tract responses are shown in Figure 4, in which they are distinguished by circles whose lower half is solid black.

Lest some devious cortical participation might conceivably still have been involved in this result, the sensorimotor area was bilaterally removed in one monkey and the entire cortex of both sides was extirpated in two cats, and movements were elicited from the bulbar pyramid. Hypothalamic stimulation was still effective in facilitating the pyramidal tract response in each case. A representative record from the cat is shown in Figure 5D and from the monkey in Figure 5E, and hypothalamic points yielding facilitation under these circumstances are indicated in Figures 2, 3 and 4 by solid black circles. In spite of the neural depression which followed acute removal of the cortex, the increased excursion of movement under these conditions (Fig. 5D and E) compared favorably in range with that obtained in the intact animal (Fig. 1), though the duration of facilitating after-discharge was never prolonged. There can be no question in these experiments but that facilitation resulted by way of brain stem connections passing downward into the cord, rather than upward to the cortex.

The fact that thalamic stimulation, particularly of the midline nuclei, facilitated cortically induced movement (Figs. 2 and 4) appears at first glance to support Murphy and Gellhorn's suggestion that the hypothalamus may excite the cortex by way of the medial thalamus (17). Further support might be derived from a single instance in this series in which hypothalamic facilitation of cortical motor response could not be obtained after extirpation of the medial portion of the thalamus, though facilitation was elicited in this animal by stimulating one side of the subthalamus (Fig. 4C, circles whose right half is solid black).

Against the partially negative evidence provided by this latter experiment are the more significant, positive observations that both the midline and intralaminar group of thalamic nuclei and the ventral nucleus of the thalamus facilitated movements induced from the pyramidal tract after cortical extirpation (Fig. 5D and E, records; Figs. 2 and 4A-C, localization). Since no independent system of connections descending from the thalamus into the lower brain stem has been established, it would appear that this thalamic facilitation of movement resulted by way of the sub- or hypothalamus.

The general conclusion to which these results point is that diencephalic stimulation facilitates cortically induced movements, not at the cortex but

within the spinal cord, to which its influence is conducted by connections descending through the lower brain stem.

*Sensory facilitation of cortical motor response.* Instances of sensory facilitation of cortically induced movement, first noted by Bubnoff and Heidenhain (2) and more recently investigated by Gellhorn and Thompson (4), were encountered in the present study. Increase of cortical motor response induced by pinching the toes is illustrated in Figure 5F. Facilitation similar to that shown in Figure 1 was obtained by stimulating the lateral spinothalamic tract and medial lemniscus in the pons of the monkey (Fig. 3B), and the medial lemniscus in the midbrain of the cat (Fig. 4D). In the record shown in Figure 5G, the response to stimulation of the leg area of the monkey's cortex was facilitated by exciting the bulbar gracile nucleus and a convulsion limited to the responding leg ensued.<sup>7</sup> It remains to be determined whether this sensory facilitation of cortically induced movement occurs entirely within the cortex, for it is possible that the brain stem system outlined above might also be excited at the diencephalic level.

### DISCUSSION

The method of study of extrapyramidal function which has been employed in the present work was inaugurated by Mettler, Ades, Lipman and Culler (15), who first studied the influence of exciting subcortical structures upon movement initiated from the cortex. While their attention was directed mainly to the basal ganglia, stimulation of the subthalamus, the centrum medianum of the thalamus and the anterior midbrain was observed to facilitate cortically induced movement. Mettler and Mettler (16) subsequently noted accentuation of cortical motor response from stimulating in and adjacent to restricted portions of the caudate nucleus (see also Fig. 3A and 4B), and in the present study such facilitation has also been obtained from activating part of the globus pallidus and the ansa lenticularis arising from it. When these observations are coupled with the demonstration by Ranson, Ranson and Ranson (21) of the ending of part of the pallidal outflow in the sub- and hypothalamus, it appears more than likely that the ventral diencephalic facilitatory system receives functional contributions from the basal ganglia.

This sub- and hypothalamic facilitatory system, first detected by Mettler, Ades, Lipman and Culler (15) and more intensively investigated by Murphy and Gellhorn (17) and in the present study, appears, as has been remarked, to exert its influence at the spinal rather than the cortical level. An uninterrupted continuity of facilitatory sites can be traced from the diencephalon backward into the lower brain stem, and diencephalic sites whose stimulation facilitates cortically induced movement also effectively

<sup>7</sup> Convulsions also sometimes resulted during or immediately after stimulation in sensory pathways at more anterior levels of the brain stem and in the ventral thalamic nucleus; they were, in addition, occasionally obtained from stimulation of the brain stem tegmentum or reticular formation at sites removed from any known sensory connections.

facilitate motor activity evoked from the bulbar pyramid, even after cortical extirpation. It is important to realize that most of the brain stem sites whose stimulation has facilitated cortically induced movement in the present experiments will themselves initiate motor activity upon stronger stimulation. A comprehensive account of the potentialities of the diencephalon for somatic motor activity has been presented by Hinsey (5), and the motor capacities of the lower brain stem tegmentum have been made evident by Ingram, Ranson, Hannett, Zeiss and Terwilliger (6).

An electro-physiological analysis of the spinal events which result from activating descending brain stem connections at the medulla oblongata has been undertaken by Lloyd (10), who finds that "impulses from long fibers reach spinal motoneurons directly and by appropriate propriospinal relays. . . . With repetitive volleys descending the cord, the internuncial activity is synchronized and augmented, and the activity so changed determines the size and duration of the motoneuron discharge." In a review of this and related work, Lloyd (11) discusses the possibility that this rapidly conducting system descending from the brain stem may set the spinal stage for cortically induced impulses arriving over the pyramidal tract.

Because it is very probably these known motor mechanisms of the brain stem whose subliminal stimulation has facilitated cortically induced movement in the present experiments, and in those of Murphy and Gellhorn (17), the question may be raised—does this facilitation simply represent the chance interaction of two motor connections thrown into activity together in an artificial and unphysiological manner, or does the brain stem normally play a significant role in providing a background of excitement in, and so preparing the spinal outflows, through which the motor cortex operates, for ready discharge?

The very fact that the pyramidal and brain stem facilitatory systems can be demonstrated to interact seems of importance, and more definite indication that the second of these possibilities may be correct, is provided by observations of motility after injury to portions of the brain stem. The effect on motor activity of sub- and hypothalamic lesions has been studied in both the cat and monkey. Because the papers describing these results have been concerned chiefly with other topics, extracts may be quoted from the protocols of two monkeys with hypothalamic lesions, observed by Ranson (19):

"Monkey 20. (After operation) the face remained immobile and mask-like. . . . (Other) activity was greatly reduced and slowed. . . . The lack of motor initiative persisted and was clearly in evidence on the forty-seventh day and although the monkey could, when activated, run, jump and climb in a normal manner, its movements did not have the extreme rapidity characteristic of those of a normal monkey.

"Monkey 25. (After operation) the face had a fixed, sad expression, which did not change. . . . There was a loss of motor initiative and slowness of movement, but not other impairment of motor control. The monkey could walk, jump and climb. It would remain in one place, without changing its position, for a considerable period. On the fifth day the cage was left with the door open and unguarded and the animal was watched for five minutes. Except for opening and closing its eyes, the monkey hardly moved. Once it shifted

its head a little. Beginning with the seventh day activity increased, but thirty days after operation movements still were slower than normal."

The lesions in these and other similar cases (19) destroyed the lateral hypothalamus and the adjacent subthalamus at the caudal end of the diencephalon. Through sensory and motor connections with the cerebral cortex were uninjured; almost no thalamic injury was present in the second case. A similar akinesia has been observed in cats with large caudal hypothalamic lesions (1, 8). A mask-like face and slowness and poverty of movement, suggestive of the Parkinsonian syndrome in man, have recently been shown by Richter (22) to follow large lesions limited with an exquisite nicety to the globus pallidus in carbon disulfide poisoning in monkeys. Kennard and Fulton (9) had previously observed that after bilateral lesions of the putamen and globus pallidus of monkeys "all movements were greatly slowed." Such pallidal lesions must evidently be extensive to produce signs, for no motor alterations resulted from fairly large lesions of the globus pallidus in the monkeys studied by Ranson and Berry (20).

It may be suggested that the hypokinesia which has followed this experimental destruction of the globus pallidus and ventral diencephalon results from impairment of the brain stem facilitatory system outlined by the present study.

#### SUMMARY

In cats and monkeys, cortically induced movements are facilitated by exciting a ventral diencephalic mechanism (sub- and hypothalamus) which appears to receive functional contributions from the globus pallidus and the midline and other nuclei of the thalamus.

An uninterrupted continuity of facilitatory sites may be followed from the ventral diencephalon backward through the central gray and tegmentum of the midbrain, the pontile tegmentum and the bulbar reticular formation.

Ventral diencephalic sites, whose stimulation facilitates cortically induced movement, are also effective in facilitating motor activity evoked from the bulbar pyramid, even after cortical extirpation.

From these results, diencephalic stimulation would appear to facilitate cortically induced movement not at the cortex but within the spinal cord, to which its influence is conducted by connections descending through the lower brain stem.

Impairment of this brain stem facilitatory system may be responsible for the hypokinesia, resembling that of Parkinson's disease in man, which follows experimental destruction of the globus pallidus and ventral diencephalon.

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# PROPERTIES OF MAXIMAL SEIZURES, AND THEIR ALTERATION BY ANTICONVULSANT DRUGS AND OTHER AGENTS†

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## INTRODUCTION

DESPITE the high incidence of convulsive disorders and widespread use of shock therapy in the major psychoses, relatively few investigations have dealt with the physiological properties of experimental seizures (1, 2, 3, 10, 12, 14, 16, 17). Convulsive threshold has received considerable attention, particularly in the testing of anticonvulsant drugs (11, 13, 15, 18, 21, and others), but threshold is only one of many properties which might be examined in any excitable system. Furthermore, measurements of threshold are of but limited value in the detection of antiepileptic potency (5, 6, 7, 8, 19, 20). For example, diphenylhydantoin, which has been found superior to phenobarbital in the treatment of psychomotor epilepsy and of comparable value in grand mal, is relatively ineffective in raising the threshold either for metrazol or electroshock seizures in laboratory animals.

Therefore we have undertaken a series of studies on other properties of experimental seizures (7, 8, 19, 20, 23). Several new techniques for the testing of anticonvulsant drugs have already evolved from these studies (7, 8, 20, 22). The technique which to date has shown the best correlation with clinical antiepileptic efficacy is based upon the ability of certain drugs to alter the character of major seizures produced by supramaximal electroshock stimulation. The present report deals with some elementary properties of maximal seizures in normal animals, and with the effects of anticonvulsant drugs upon these properties.

## METHODS

Seizures were produced in cats, albino rabbits, and Sprague-Dawley rats by an Offner clinical electroshock apparatus delivering from 0 to 700 milliamperes (mA.) of 60 cycle alternating current with stimulus duration of 0.05 to 10.0 sec. For small current values an external attenuator was added. Current during stimulation was checked with an external peak AC milliammeter. For shocks of longer duration a variable transformer was used. Shocks were usually delivered through Spiegel corneal electrodes (18). In some rabbits the stimulating electrodes were insulated steel screws aseptically implanted over the visual and motor areas of one cerebral hemisphere. In these animals similar electrodes over the opposite hemisphere were used for recording of EEG seizure discharges, the visual and motor areas being compared with an "indifferent" electrode in the nasal bone; the EEG was recorded with a Rahm E2X60 two-channel thermal-writing electroencephalograph

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with time-constant of 0.2 sec. and flat frequency-response characteristic up to 40 cycles per sec.

For determinations of seizure threshold, shocks of 0.2 sec. duration were given at intervals of 5 or more minutes with successive increments of 10 per cent in current until a "minimal" seizure occurred. This "minimal" seizure, which was shown in rabbits to be the least overt activity accompanied by EEG seizure discharges, consisted of 10 or more seconds of facial clonus without loss of righting reflexes. At least several hours were permitted for recovery following each seizure, except in those cases where the recovery period itself was investigated. "Submaximal" seizures of increasing severity were produced by successive small increments of current above threshold. The "submaximal" seizures consisted either of generalized clonus, or contained a preliminary tonic phase with flexion at limb joints. In either case postural reactions were lost during the seizure. When further increments of current failed to alter the pattern or duration of seizures, they were considered to be "maximal" seizures. They usually consisted of a short flexor and long extensor tonic component with little or no terminal clonus. All strengths or current above that required to produce them in normal animals were considered "supramaximal." In most animals the "supramaximal" level began not more than 20 per cent above threshold. When it was necessary to produce unequivocal "maximal" seizures for studies of recovery or drug action, the stimulating current was arbitrarily set at least 200 per cent above the normal seizure threshold.

In evaluating the effect of antiepileptic drugs the following simplified procedure was used ("supramaximal electroshock method"). In each determination a series of animals was treated with a range of doses up to the toxic level. After a suitable interval for optimal drug action, the neurological status of each animal was examined. Immediately afterward corneal electrodes were applied and a supramaximal shock delivered. Animals were observed for presence or absence of an extensor tonic component in the hindlegs during the seizure. The protective dose of the anticonvulsant agent was taken to be that required to abolish this extensor tonic component. The toxic dose was taken to be that causing minimal signs of central impairment. A protective index was calculated for each agent by dividing the toxic dose by the protective dose. Antiepileptic agents studied by this method included sodium diphenylhydantoin (dilantin),<sup>1</sup> sodium phenobarbital, 3,5,5-trimethyl-oxazolidine-2,4-dione (tridione),<sup>2</sup> dimethyl-N-methyl-barbituric acid (AN 22),<sup>2</sup> diethyl-N-methyl-barbituric acid (AN 23),<sup>2</sup> benzimidazole, sodium bromide, and l(+) glutamic acid. Other details of technique will be found under Results.

## RESULTS

*Evidence indicating that tonic extensor seizures are maximal.* Tonic extensor seizures were elicited in most rabbits by shocks not more than 20 per cent above threshold for minimal seizures. The only consistent trend noted with increasing current was a decreased latency. Thus in six animals the average latent period before development of flexor tone was reduced from six sec. at 75 mA. to two sec. at 300 mA. The total duration of seizure was correspondingly reduced with increasing strength of current. In 108 seizures elicited in 50 albino rabbits stimulated for 0.2 sec. with 300 mA. (or approximately six times the threshold current for minimal seizures), the following components of the convulsion were relatively invariable in character, sequence and duration: (a) *Latent period.* Approximately two seconds. (b) *Flexor component of tonic phase.* Extreme tonic flexion at all limb joints with slight superimposed tremor, lasting approximately three seconds. (c) *Extensor component of tonic phase.* Extreme extension at all limb joints with

<sup>1</sup> Generously supplied by Dr. Oliver Kamm of Parke, Davis and Co.

<sup>2</sup> Generously supplied by Dr. R. K. Richards of the Abbott Laboratories.

little or no tremor, followed by abrupt relaxation; average duration 14 seconds. The average elapsed time from stimulation to end of the tonic phase was 19 seconds (range 15 to 25 sec.,  $SD \pm 2$  sec.). (d) *Clonic phase (frequently absent)*. One or more extensor thrusts, followed by complete relaxation; average duration two seconds. (e) *Period of post-seizure depression*. Inability to exhibit contact placing reactions and to maintain a second maximal seizure; average duration four minutes.

In two out of 52 albino rabbits, and in four out of seven colored rabbits, it was impossible to produce the extensor tonic component, even though the current was increased to 500 mA. Such animals were avoided in assays of anticonvulsant drugs.

In rats and cats the seizure pattern was identical with that in rabbits except for the time scale. In 50 control rats, tested with supramaximal shocks of 150 mA. and 0.2 sec. duration, or approximately five times the threshold, the average elapsed time to the end of tonic seizures was 14 seconds (range, 12 to 17 sec.,  $SD \pm 1$  sec.). In two additional rats, flexor tonic seizures only were elicitable. Twenty-four cats were tested with 400 mA. for 0.2 sec., or approximately five times threshold. The mean duration to the end of the extensor tonic component was 15 sec. (range, 10 to 20 sec.,  $SD \pm 3$ ). In four additional cats, only flexor tonic seizures were exhibited.

The following procedures failed to increase the duration or severity of tonic extensor seizures in rabbits and rats: variation in electroshock current from 20 per cent above threshold to 10 times threshold; variation in shock duration from 0.2 to over 30 sec.; stimulation by additional supramaximal shocks at various times during the course of a seizure; reduction in electroshock threshold by metrazol or by cellular hydration. It would therefore appear that the brain is maximally active during a tonic extensor seizure, and the discharge once initiated is independent of the stimulus.

*Modification of maximal seizures by repetition.* Thresholds were re-determined in rabbits at various intervals following maximal seizures. They were found to average 200 per cent of normal at 4 minutes, and 118 per cent at 60 minutes, so that the period of increased threshold considerably outlasted any overt neurological signs of post-seizure depression. Maximal seizures could be elicited again as early as four minutes after a previous seizure (*i.e.*, when the threshold had fallen to 200 per cent of normal). However, continued repetition of seizures resulted in a progressive slowing of recovery and a modification of the various components of seizures in the following order: (a) The first effects were an increased duration of flexor tonic component, a reduced duration of the hindleg extensor tonic component, a decrease in total tonic phase, an increase in duration of the clonic phase, and an increase in total seizure duration. If the tonic flexor component persisted beyond approximately 10 sec., no tonic extension developed. The tonic flexor component itself did not persist beyond 15 sec. (b) With further elicitation of seizures the tonic flexor component was shortened until it also

disappeared. The seizure was now completely clonic, often severe, and frequently longer in duration than that of the unmodified maximal seizure.

(c) With still further elicitation of seizures, the clonic type of convulsion became shorter and milder and sometimes was abolished completely. (d) Following a mild clonic seizure or a complete failure to respond, the next shock in such a series often produced a severe submaximal convulsion. Thereafter the responses tended to alternate between mild and severe.

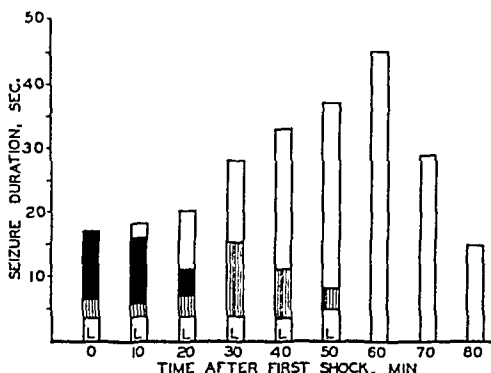


FIG. 1. Effect of repeated electroshock on seizure pattern in a rabbit stimulated with shocks of 100 mA. (twice threshold) and 0.2 sec., repeated at 10-minute intervals. Black: extensor component of tonic phase. Barred: flexor component of tonic phase. White: clonic phase. "L": latent period (not measured in purely clonic seizures).

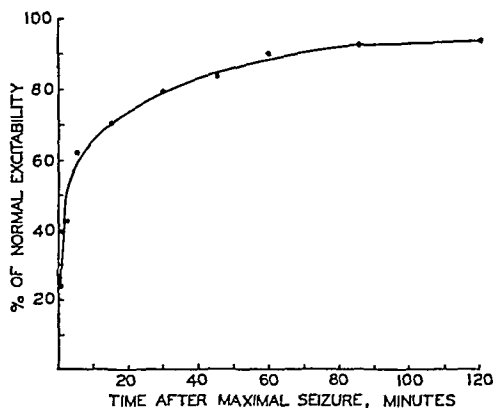


FIG. 2. Rate of recovery of electroshock threshold following maximal seizures in normal rats.

The progressive effects of repeated supramaximal electroshock are illustrated in Figure 1. The time course of recovery of seizure threshold following maximal convulsions was studied in normal rats and found to be similar to that in rabbits. The results are illustrated in Figure 2. The recovery period was also investigated in rats in which cellular hydration produced by extracellular electrolyte depletion (20) had reduced the seizure threshold by more than 50 per cent. In spite of the difference in threshold, the time course of recovery was identical in both normal and hydrated animals, indicating the relative independence of the excitation and recovery processes.

As might be expected, thresholds in both rats and rabbits were found to be greater after maximal seizures than after clonic seizures elicited by shocks just above threshold. This was true even though the clonic seizures were usually longer in duration than the maximal seizures.

In rabbits in which the tonic extensor component had been abolished by repeated supramaximal shocks at intervals of ten minutes or less, the complete maximal seizure pattern could often be restored by increasing the shock strength or duration, or by delivering additional shocks during the course of a clonic or tonic flexor seizure. During submaximal seizures, the brain is therefore capable of reinitiating and sustaining maximal seizures.

*Effect of anticonvulsant drugs on maximal seizures.* The alterations produced by anticonvulsant drugs were similar to those following repeated seizures in untreated animals. Progressive changes in the character of seizures with increasing doses of diphenylhydantoin are illustrated in Figure 3.

Table 1. Relative efficacy of various agents in modifying maximal electroshock seizures†

Species	Agent	Route	Protective Dose mg./kg. (P)	Toxic Dose mg./kg. (T)	Protective Index (I = T/P)
Rat	Diphenylhydantoin	i.p.	50	100	2.0
Rabbit	"	s.c.	60	180	3.0
Cat	"	i.p.	10	40	4.0
Rat	Phenobarbital	i.p.	12	30	2.5
Rabbit	"	i.p.	15	35	2.3
Cat	"	i.p.	2	5	2.5
Rat	Pentobarbital	i.p.	12	14	1.2
Rabbit	"	s.c.	8	12	1.5
Cat	"	i.p.	3	3	1.0
Rat	Tridione	i.p.	350	400	1.1
Rabbit	"	i.p.	500	875	1.7
Cat	"	i.p.	200	300	1.5
Rat	Benzimidazole	i.p.	50	50	1.0
Rabbit	"	s.c.	135	180	1.3
Cat	"	i.p.	100	50	0.5
Rat	AN 22	i.p.	200	200	1.0
Rabbit	"	i.p.	250	350	1.4
Cat	"	i.p.	125	125	1.0
Rat	AN 23	oral	300	250	0.8
Rabbit	"	i.p.	200	200	1.0
Cat	"	oral	15	35	2.3
Rat	Glutamic Acid	i.p.; oral	*	3000	0
Rabbit	"	i.p.	*	1500	0
Cat	"	i.p.; oral	*	8000	0

† Current strength: rabbits 300 mA., cats 400 mA., rats 150 mA. Current duration: 0.2 sec.

P = dose required to abolish extensor tonic phase of seizure induced by supramaximal electroshock.

T = dose required to produce signs of central impairment.

\* Failed to alter seizures in any dose.

The ability of several agents to modify maximal seizures is summarized in Table 1. In all three species, diphenylhydantoin and phenobarbital were effective in doses 50 per cent or less of that required to produce signs of central impairment (ataxia, loss of placing reactions, etc.). Tridione, pentobarbital, benzimidazole, AN 22 and AN 23 were all effective, but their protective indices were lower. In preliminary observations in rabbits, ether

and sodium bromide were found effective but only in depressant doses. 1(+) glutamic acid was completely ineffective in all animals even at lethal doses.

The purely clonic seizure produced by supramaximal electroshock in an animal protected by diphenylhydantoin was almost always longer in duration

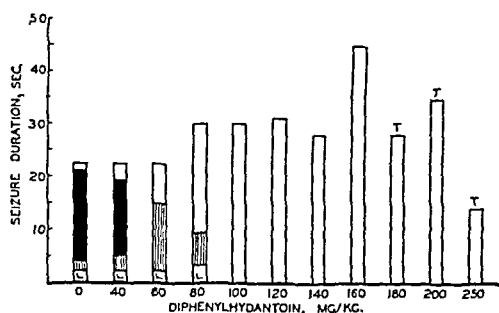


FIG. 3. Effect of diphenylhydantoin on seizure pattern in rabbits. Series of 11 animals treated five hours previously with the indicated subcutaneous dosages of diphenylhydantoin. Key as in Fig. 1. "T" = toxic dose (signs of central impairment noted just before shock). Shocks of 300 mA. and 0.2 sec.

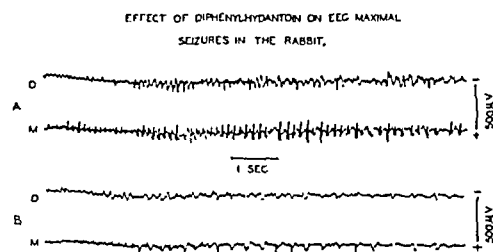


FIG. 4. Effect of diphenylhydantoin on EEG response to supramaximal electroshock in the rabbit. A: control. B: after 100 mg./kg. of sodium diphenylhydantoin, s.c. "O": occipital, and "M": motor epidural electrode placements, paired with "indifferent" electrode in nasal bone. Records begin four sec. after electroshock. Note reduction in amplitude of surface-negative spikes and slowing of frequency after diphenylhydantoin.

recorded from the motor cortex, and a general slowing of frequency of discharge. This last-named effect was seen with other agents capable of modifying maximal seizures.

Experiments in rats (20) as well as in cats and rhesus monkeys (7, 8) have also demonstrated the inability of diphenylhydantoin to raise the

duration than the control maximal tonic seizure in the same animal. Prolonged and violent clonus often continued for a minute or longer. Yet the central impairment in the post-ictal period was less severe and the rate of recovery more rapid in the diphenylhydantoin-treated animals. The alteration of seizure pattern by diphenylhydantoin was of particular interest because of the inability of this drug to increase the electroshock threshold for minimal seizures in any dosage. For example, electroshock thresholds in the same rabbits as shown in Figure 3 remained within the control range of 45 to 65 mA. after treatment with 100 mg./kg. of diphenylhydantoin and no individual animal showed an increase over its control level. Similar observations over a wider dosage range were made in five additional animals whose EEG's were recorded from implanted epidural electrodes. No significant alteration occurred in the EEG seizure threshold. This was true for long (9 sec.) as well as for short (0.2 sec.) duration of electroshocks. However, the EEG seizure pattern following supramaximal shocks was altered toward a more clonic type, as shown in Figure 4, with a reduction particularly in the surface-negative spikes

normal seizure threshold, although this agent in non-depressant doses protects against the tonic phase of seizures in all species. Diphenylhydantoin was found to modify maximal seizures in rats even when seizure threshold was reduced by cellular hydration. An additional point of interest revealed in the cat experiments was the prolonged duration of action of single doses of diphenylhydantoin. Doses of 20 mg./kg. abolished tonic seizures for an average of seven days (seven cats) while 40 mg./kg. extended the period of protection to 11 days (six animals). Other agents even in large doses failed to show such prolonged action. Diphenylhydantoin protection lasting for more than a day was also noted in rabbits and rats.

The most convenient animals for assay of anticonvulsant drugs were found to be rats of the Sprague-Dawley strain because of their docility and uniformity of response.

### DISCUSSION

Rosenblueth and Cannon (17) reported a direct relation between seizure duration and "quantity" of stimulation. Their observations were made on chloralose-anesthetized animals. If such a relation exists in normal unanesthetized animals, it must hold true for only a portion of the range of stimulus strength. Tonic extensor seizures are of relatively constant duration for the individual animal over a wide range of stimulus conditions. Such maximal seizures can be elicited in most animals by shocks not far above threshold. The present studies indicate that during a tonic extensor seizure all neuronal circuits capable of contributing to the discharge are maximally active, and that the seizure cannot be further modified once it has begun. It would seem that the brain, like the individual neurones of which it is composed, is normally capable of responding in an all-or-none manner.

The constancy in form and duration of maximal seizures in normal animals suggests a fixed quantity of energy expended in each cerebral "explosion." With antiepileptic therapy this quantity of energy may be reduced, as indicated by the more rapid recovery from the purely clonic type of seizure following diphenylhydantoin treatment, while at the same time the dissipation of this energy may be spread out over a longer period, as indicated by the greater duration of such clonic seizures.

Clinically established anticonvulsants (diphenylhydantoin, bromide, phenobarbital, tridione) have in common the ability to abolish the tonic extensor component of a maximal seizure produced by a brief electroshock several times greater than the normal threshold current. This property is particularly significant with respect to diphenylhydantoin which is ineffective in altering the normal electroshock threshold or in protecting against the standard convulsive dose of metrazol. The results reported suggest the possibility that the efficacy of clinical antiepileptic agents may be better correlated with a reduction in the ability of the brain to support self-sustaining discharges than with a simple increase in the electrical or chemical thresh-



hold for initiation of such discharges. Not all investigators are agreed that the electroshock seizure threshold in epileptic patients is abnormally low (4, 9, 14).

As a procedure for laboratory identification and assay of antiepileptic drugs, the supramaximal electroshock method possesses the following advantages over more usual techniques involving threshold determinations: (i) The ability of the brain to maintain a maximal seizure is studied independently of the threshold for minimal seizures. (ii) The endpoint chosen is sharp and easily recognized, whereas threshold determinations involve an element of judgment between mild seizures and simple hyperactivity unless EEG tracings are simultaneously taken. (iii) Only one electroshock is administered per animal per determination, thus eliminating the effect of subconvulsive shocks or alterations due to unrecognized minimal seizures.

In spite of these advantages it is felt that this method should supplement rather than displace other techniques. The simultaneous use of several independent criteria may yield information on the different mechanism of action of particular antiepileptic agents and thereby help to differentiate the neurological and metabolic disturbances underlying the several clinical types of convulsive disorders.

#### SUMMARY

1. Seizures produced in rabbits, cats, and rats by electroshock intensities not far above threshold are usually characterized by extreme tonic extension, and are relatively constant in duration. This tonic extensor type of seizure is not altered by further increase in stimulus intensity or by lowering of threshold. Once it has begun it cannot be modified by additional stimulation while in progress. When the tonic extensor component is abolished by repeated electroshock, it may be restored by stimulation during a seizure. The depression following tonic extensor convulsions is uniform in duration and greater than for purely clonic seizures, although the latter are often considerably longer. The tonic extensor seizure would appear to represent the maximum rate of dissipation of energy of which the brain is capable.

2. The clinically recognized antiepileptic agents abolish the tonic phase of major seizures even when these drugs fail to raise appreciably the threshold for electroshock or metrazol seizures. Diphenylhydantoin and phenobarbital show the highest protective index. Several new agents including tridione rank with pentobarbital in efficacy.

3. A rapid and simple method for detecting and evaluating experimental antiepileptic agents is presented.

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# TRANSMISSION OF IMPULSES IN PERIPHERAL NERVES TREATED WITH DI-ISOPROPYL FLUORPHOSPHATE (DFP)

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## INTRODUCTION

RECENT developments regarding the role of acetylcholine and cholinesterase in the physiology of the nervous system have included suggestions as to the possible importance of these substances in the conduction of the nerve impulse along the nerve fibers. Consequently, not only must the physical theory of nerve conduction which has received additional support by the experiments of Hodgkin (13) and of Tasaki (27) be considered, but a new set of observations relating to the possible involvement of acetylcholine must be kept in mind. The latter observations began with the experiments of Calabro in 1933 and of Bergami in 1936 (citations from 16), who observed the liberation of acetylcholine in nerve trunks following stimulation. Subsequent workers (7, 15, 28) noted that although resting nerves contain acetylcholine, stimulation of the nerves leads to an increased output of this compound. Cholinesterase was demonstrated to be present in nerve fibers and was shown to be concentrated at the surface rather than in the axoplasm (4, 5, 26). Thus was established the presence in approximately the proper location of the enzyme which is known to destroy and thus to localize the action of acetylcholine. Next followed the observation that degeneration of nerve fibers leads to a decrease in the content of cholinesterase (23). The presence of choline acetylase in nerve fibers and the demonstration of a decrease in the activity of this enzyme following degeneration of nerve fibers was also demonstrated (24, 25). Meanwhile a parallel relationship was proposed between the cholinesterase content of the electric organ of the electric eel and the voltage developed by this organ (21, 22, 26). The suggestion that acetylcholine possesses electrogenic activity was made by Beutner and Barnes (2) in an experiment which showed the power of this drug in producing electronegativity in a physico-chemical system containing lipoids. With some of these data as a background, Fulton and Nachmansohn (10) placed on a formal basis the conception that acetylcholine may have a primary role in the production of the electrical changes occurring at the surface of neurones. Thus in the brief span of a dozen years was developed a set of observations pointing suggestively toward acetylcholine as a participant in the mechanism responsible for the production of the nerve action potential. Critical, opposing or doubting views have periodically appeared. Gaddum, Khayyal and Rydin (11) interpreted their findings to indicate that acetylcholine was liberated from nerve trunks only under conditions of excessively

strong stimulation or when damage was involved, while no evidence of acetylcholine liberation in purely sensory nerves was obtained by Loewi and Hellauer (17). Cowan (8) was unable to obtain any effect on nerve fibers by soaking them in solutions of eserine or eserine-like compounds and Lorente de N6 (18) reported no action for acetylcholine on the conduction of nerve impulses. The latter result was confirmed on single nerve fibers by Hertz (12). By the in vivo administration of eserine into frogs, Cantoni and Loewi (6) were unable to find evidence of impaired conduction of impulses in the peripheral nerves of such eserinizied animals. A number of arguments against the thesis that acetylcholine is causally related to the nerve action potential have been advanced by Feldberg (9). Thus it appears that not all interpretations are in harmony with the view that acetylcholine possesses a primary role in the process of conduction so that further elucidation of the relation between acetylcholine and the action potential is essential. Of special interest would be information that would clarify the relation between acetylcholine concentration in nerve fibers and the magnitude of the action potential.

A compound which affords a fresh approach to the above problem is diisopropyl fluorophosphate (DFP). This drug has been shown to have an irreversible anticholinesterase action (14, 19, 20). With this compound it should be possible to inactivate irreversibly all the cholinesterase in a nerve. Such a nerve would then offer a unique opportunity to test the relationship between cholinesterase and the conduction of nerve impulses. It is assumed, in connection with the concept of the primary role of acetylcholine in conduction, that the absence of cholinesterase would, with continued activity, lead to a concentration of acetylcholine at the active surface and would result eventually in an impairment of conduction. A nerve without cholinesterase activity might show (i) no effect on nerve conduction, (ii) immediate cessation of conduction, or (iii) gradual block of conduction as acetylcholine accumulates with continued stimulation. If the first result were obtained the concept of a primary role for acetylcholine and cholinesterase in the conduction of nerve impulses would be seriously weakened, while if either the second or third result were to appear, this concept would receive definite support. In the present experiments it will be demonstrated (i) that nerves which are virtually free of cholinesterase can be obtained by the use of DFP and (ii) that such nerves conduct in essentially the same manner as normal nerves in response to both single and repetitive stimulation.

#### METHODS

Two series of experiments were performed. The first was an in vitro series in which isolated nerves of the bullfrog (*Rana catesbiana*) and of the cat were mounted in a moist chamber placed in a constant temperature bath. Silver wires, arranged as shown in the diagram of Figure 3E, were used as stimulating and lead-off electrodes. In the diagram, C and A represent, respectively, the cathode and anode of the stimulating couple, the point G was grounded and D represents a small dish into which a 1.5 cm. section of nerve was looped and into which was placed any solution whose effect on the nerve was to be tested. Points numbered 1, 2 and 3 were used as lead-off points for the action potentials. Electrode 1, when pitted against the KCl-treated section (electrode 3), served as a monophasic lead

(lead 1) at a point proximal to the dish region. This lead was useful as a check on the condition of the nerve throughout the experiment. Electrode 2, when pitted against the KCl-treated portion (electrode 3) served as a monophasic lead (lead 2) distal to the dish. This lead was used to test the effect of the solution on conduction. Electrode 1, when pitted against electrode 2, gave a diphasic lead (lead 3) also useful in checking the effect of any blocking agent applied at D, since a block of impulses at D should result in monophasicity in lead 3. The stimulus was obtained by means of a thyatron stimulator furnishing either single or repetitive shocks of variable strength. The form of the stimulus was essentially that of a condenser discharge with a rate of discharge such that the 50 per cent level was attained in 200 microseconds. The action potentials were led off to a push-pull amplifier and recorded by means of a cathode ray oscillograph. The single sweep was tripped by means of the stimulator. In this first series of experiments, performed on 25 frog nerves and 5 cat nerves, information was obtained on the effect of DFP when applied locally to a nerve *in vitro*. The action of DFP was compared to that of eserine and of eserine salicylate.

The second series of experiments was carried out on 22 bullfrogs. Into each of 11 of these were injected 2 grams of DFP per kilogram by way of the ventral lymph sac. At the end of 1-2 hours, when the frogs were completely or nearly completely paralyzed, the two sciatic nerves along with the tibial branches were carefully dissected out and placed in the moist chamber in the same manner as in the first series of experiments with the exception that the dish at D was omitted. Two nerves from a control frog were similarly removed and the action potentials of the experimental and control nerves were photographed and compared. For each experimental frog, a control was used on the same day. At the end of the period of recording the two experimental nerves were combined, washed in Ringer solution, homogenized with powdered silica in a Potter-Elvehjem homogenizer in a solution containing 4 parts (by volume) of 0.04 M  $MgCl_2$  and 1 part of 0.15 M  $NaHCO_3$ . The dilution was such that 1 ml. of homogenate was equivalent to 100 mgs. of nerve. The cholinesterase content was then determined at 25°C. on 1 ml. of the homogenate using Warburg manometers with small (5 ml.) flasks. Complete details of the technique are included in the reports of Mazur and Bondansky (20) and of Koelle and Gilman (14). The  $CO_2$  liberation was followed for an hour at a pH of 7.4. The 2 control nerves were similarly treated and their cholinesterase activity estimated simultaneously with that of the experimental nerves. To eliminate any question regarding the possibility of the presence of free DFP in the homogenate from the experimental nerves, which would interfere with the determinations, a control was set up as follows. To 0.5 ml. of the homogenate from the experimental nerves was added 0.5 ml. of the homogenate from the control nerves and the cholinesterase content of this mixture was measured. As Table 1 (column 6) indicates, a recovery close to the expected value (one half the sum of the activities of the control and experimental homogenates) was obtained, within the standard error of the method. Thus not enough DFP was carried over into the reaction vessels to interfere with the measurements.

## RESULTS

*Series I.* When eserine salicylate (0.01-0.02 M) in Ringer solution was added to the dish at D no effects on the action potentials were recorded up to 1-2 hours after the addition of this compound. This is demonstrated by the experiment on frog nerve in Figure 1 (column C) in which the compound action potential for lead 2 before the addition of eserine salicylate (record 1), and 60 minutes after the addition of this compound (record 2) are compared. There is no apparent difference in either amplitude or wave form in these two electrograms. The fact that eserine salicylate produced no effect served as a control both on the addition of Ringer solution to the nerve and on possible osmotic effects. In contrast, eserine base in the same concentration produced, within a matter of minutes, a block, as revealed by the failure of impulses to reach lead 2 (Fig. 1: C-3 to C-8; D-1 to D-2). The block, however, was reversible as shown by the fact that after washing the nerve seg-

ment at D with Ringer solution, a large proportion of the potential was restored (Fig. 1: D-3). Thus of the two anticholinesterases, eserine produced a block which was at least partly reversible while eserine salicylate acting over a much longer period of time had no effect.

The addition of DFP to the dish resulted in the blocking of nerve im-

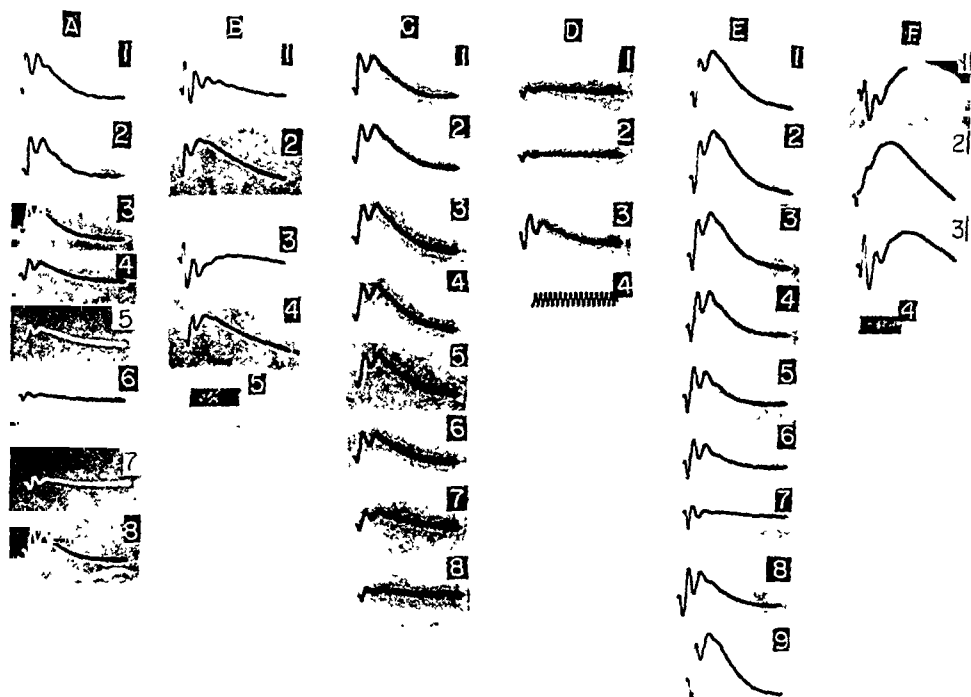


FIG. 1. Neurograms of frog nerves at 25.5°C. Stimulation was at central end of sciatic and action potentials were led off tibial branch.

Columns A, B: effect of 0.02M DFP. Lead 2 before (A-1) and 3, 6, 10, 12 and 17 minutes (A-2 to A-6) after DFP and 1 minute (A-7) and 4 minutes (A-8) after lifting nerve. Lead 1 before (B-1) and 15 minutes (B-2) after DFP. Lead 3 before (B-3) and 16 minutes (B-4) after DFP. B-5 is 1,000 cycles.

Columns C, D: effect of eserine salicylate (0.01M) and eserine (0.01M). Lead 2 before (C-1) and 60 minutes (C-2) after eserine salicylate. Lead 2 at 1, 2, 3, 4, 5, 6, 7 and 26 minutes (C-3 to D-2) after eserine and after (D-3) nerve was washed. D-4 is 1,000 cycles.

Columns E, F: effect of 0.02M DFP. Lead 2 before (E-1) and 1, 4, 6, 10, 12 and 20 minutes (E-2 to E-7) after DFP and 1 minute (E-8) and 9 minutes (E-9) after lifting nerve. Lead 3 before (F-1) and 18 minutes (F-2) after DFP and 5 minutes (F-3) after lifting nerve. F-4 is 1,000 cycles.

pulses in a matter of minutes. This occurred for both the nerves of the frog and of the cat. The photographs in columns B and C (Fig. 2) are neurograms of the cat tibial nerve and illustrate the DFP block. The control record for lead 2 before the addition of DFP was obtained with Ringer solution in contact with the nerve at D(B-1). The corresponding lead 1 Ringer control

(C-1) and lead 3 Ringer control (C-4) are also shown. The progressive action of 0.02M DFP in Ringer solution over a period of 18 minutes is demonstrated in records B-2 to B-8, which are lead 2 neurograms. During this time lead 1 (C-2) still gave a prominent action potential indicating that the nerve had not undergone progressive degeneration. Meanwhile lead 3 (C-5) lost its diphasicity and actually became identical with the lead 1 electrogram (C-2), as was expected with a block at region D. The DFP block was readily reversible, for washing the nerve segment at D with Ringer solution restored in 5 minutes (B-9) a large proportion of the potential of lead 2, while lead 3 (C-6) again showed evidence of diphasicity. Thus a compound with presumably irreversible anticholinesterase activity produced an easily reversible block when applied locally to a nerve. Another experiment with a cat tibial nerve (Fig. 2: D, E) illustrates the almost complete block in lead 2 produced in 16 minutes by DFP, a block which again proved to be reversible by washing. It soon became evident that not even washing the nerve was necessary in order to reverse the block, for equally good reversibility was demonstrated by merely lifting the nerve out of contact with the DFP solution in the dish. This is shown for frog nerve in Figure 1 (A, B) where A-1 is the lead 2 Ringer control and records B-1 and B-3 are, respectively, the corresponding controls for leads 1 and 3. The blocking effect of 0.02M DFP is pictured in records A-2 to A-6. After the block both lead 1 (which was initially somewhat diphasic) and lead 3 were completely monophasic (B-2, B-4), as expected. The nerve segment at D was then lifted out of the DFP solution and the potentials for 1 minute after lifting (A-7) and 4 minutes after lifting (A-8) showed that a large proportion of the potential was restored by this simple expedient. Another experiment (Fig. 1: E, F) showed complete reversibility of the block by lifting out the nerve and illustrated how lead 3, originally diphasic in the pre-block period (F-1), became monophasic after the block (F-2) and again diphasic when the nerve was lifted (F-3). Thus it appears that immersion of a nerve segment within a volume of DFP solution was essential for the maintenance of a block. Attempts were made to determine the cholinesterase content of the portions of the nerves exposed to the DFP, but such attempts were discontinued when it was found impossible to wash away all the uncombined DFP. It was this failure to measure the cholinesterase activity of nerves after the local application of DFP that led to the *in vivo* (series II) experiments.

*Series II.* Table 1 summarizes the cholinesterase activity of the 22 experimental and the 22 control nerves and shows that the *in vivo* administration of DFP reduced this activity to a mean level of 2.3 per cent of that in the control nerves. This figure, being well within the standard error of the method, may be considered as showing that the acetylcholine-splitting power of the nerves was reduced virtually to zero. Determinations made on nerves which had been stored in the refrigerator for 3 days after excision gave no reason to believe that the inactivation of cholinesterase became reversible during the course of the experiments.



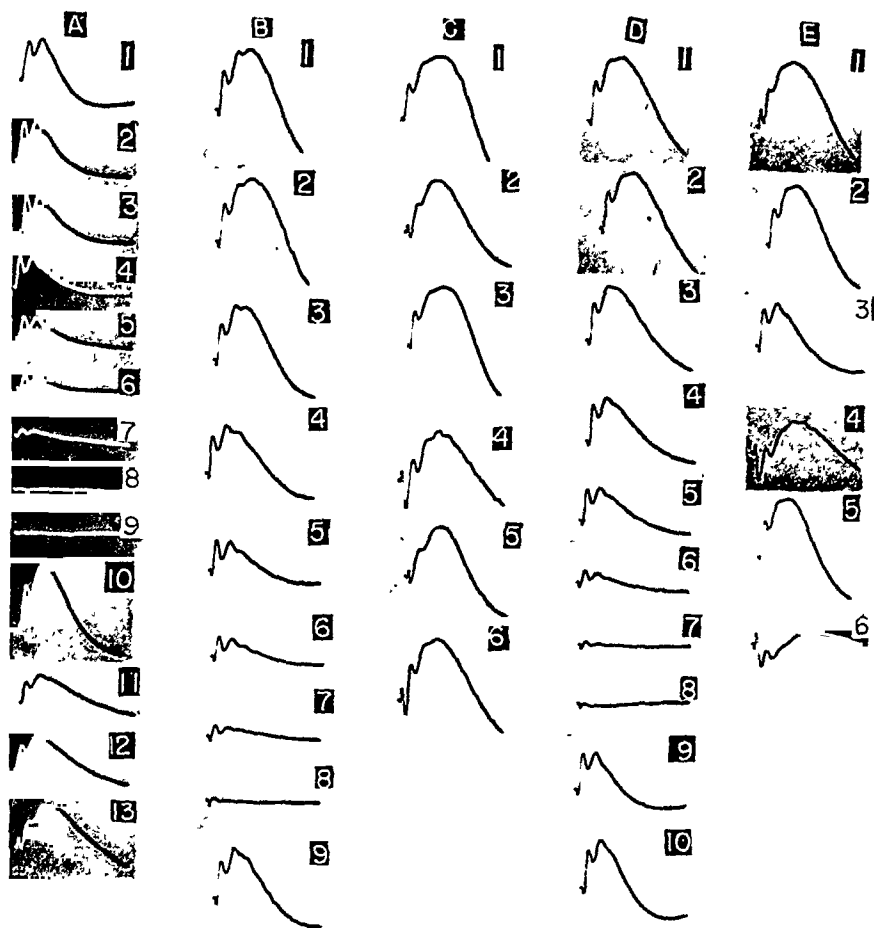


FIG. 2. Neurograms of cat nerves at 37.5°C., effect of 0.02M DFP.

Column A: peroneal nerve. Lead 2 before (A-1) and 2, 4, 5, 6, 7, 8, 9 and 10 minutes (A-2 to A-9) after DFP. Lead 1 before (A-10) and 11 minutes (A-11) after DFP. Lead 3 before (A-12) and 13 minutes (A-13) after DFP.

Columns B, C: tibial nerve. Lead 2 before (B-1) and 1, 7, 9, 12, 13, 14 and 18 minutes (B-2 to B-8) after DFP and 5 minutes (B-9) after washing nerve. Lead 1 before (C-1) and 19 minutes (C-2) after DFP and 11 minutes (C-3) after washing. Lead 3 before (C-4) and 20 minutes (C-5) after DFP and 12 minutes (C-6) after washing.

Columns D, E: tibial nerve. Lead 2 before (D-1) and 1, 5, 7, 8, 9, 10 and 16 minutes (D-2 to D-8) after DFP and 3 minutes (D-9) and 6 minutes (D-10) after washing. Lead 1 before (E-1) and 14 minutes (E-2) after DFP and 11 minutes (E-3) after washing. Lead 3 before (E-4) and 15 minutes (E-5) after DFP and 12 minutes (E-6) after washing.

A comparison of the action potentials of these nerves with the control nerves (Figs. 3 and 4) demonstrated that the lack of acetylcholine-splitting enzymes in no way impaired the ability of the nerves to conduct impulses, either to single or to repetitive stimulation. This is indicated in the neuro-

grams of Figure 3 in which the action potentials of the two control nerves from frog 4 (A, B) are compared with those recorded from the experimental nerves of frog 3 (C, D). A similar series (Fig. 4) is given for the control nerves of frog 18 (A, B) and the experimental nerves of frog 17 (C, D). The responses of these nerves after 10 minutes of repetitive stimulation at 14

Table 1. Cholinesterase activity of nerves (sciatic and tibial) of normal frogs and of frogs treated with DFP

CONTROL NERVES		EXPERIMENTAL NERVES			MIXTURE
1.	2.	3.	4.	5.	6.
Frog number	Ch. E. Activity mm <sup>2</sup> CO <sub>2</sub> /100 mg./hour	Frog number	Ch. E. Activity mm <sup>2</sup> CO <sub>2</sub> /100 mg./hour	% of mean control activity	0.5 ml. control plus 0.5 ml. experimental % expected activity
2	33.2	1	1.7	3.8	116
4	45.3	3	0.5	1.1	105
6	43.4	5	0.6	1.4	111
8	33.3	7	0.3	0.7	110
10*	37.5	9*	4.4	10.0	124
12*	44.1	11*	4.7	10.6	94
16	51.8	13	0.7	1.6	90
18	62.7	17	0.0	0.0	115
20	15.6**	19	-3.3	-7.0	94
22	45.5	21	0.0	0.0	104
24	47.0	23	1.4	3.2	110
Means***	44.4 ± 8.8 44.4 ± 2.8		1.0 ± 1.8 1.0 ± 0.6	2.3 ± 4.7 2.3 ± 1.4	106.6

Note: all experimental frogs received 2.0 gms. DFP per kg. except frog 1 which received 1.0 gm. per kg.

\* On the two days these nerves were analyzed the water-bath developed trouble leading to non-uniform temperature distribution in the bath. The thermomanometer gave an unusually high reading on both these days.

\*\* This nerve was observed to be covered with an abnormal exudate while in the frog. The action potentials developed by it were unusually low. Its cholinesterase activity has not been included in the mean figure for the control nerves.

\*\*\* The standard deviation is given as well as the standard error.

$$\text{Standard Deviation} = \sqrt{\frac{\sum d^2}{n-1}}$$

$$\text{Standard Error} = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

shocks per second are given in records 9 (Fig. 4). It is evident from these oscillograms that the action potentials of nerves from DFP treated frogs were not significantly different from those of control nerves which had their normal quota of cholinesterase. Repetitive stimulation for 10 minutes with 43 shocks a second gave no indication of a significant difference in the responses of normal and experimental nerves.

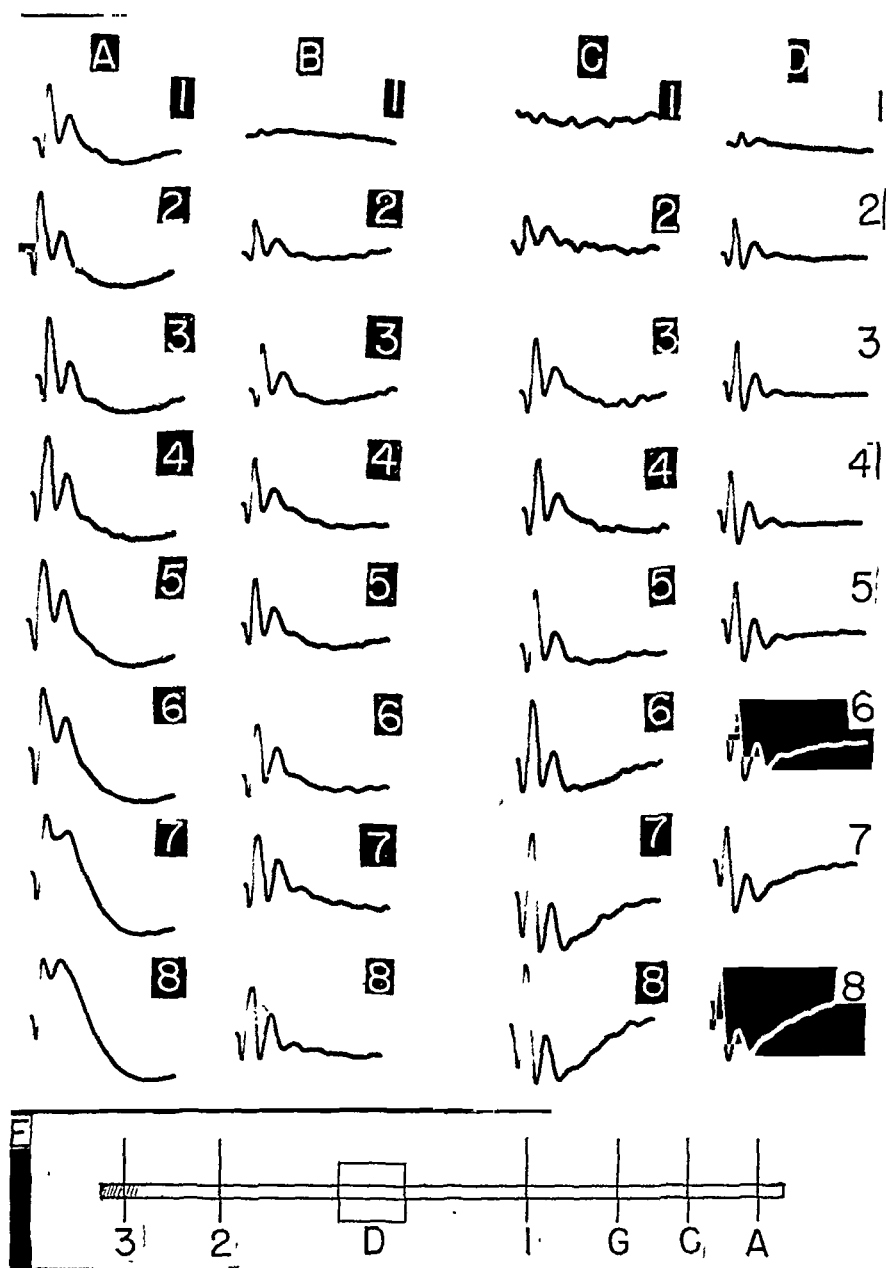


FIG. 3. Action potentials to single stimuli of increasing strengths (1-8) from the sciatic-tibial nerves of control frog 4 (A, B) and experimental frog 3 (C, D). Relative strengths of stimuli for records 1-8 were: 1, 1.2, 1.3, 1.7, 2.0, 2.5, 3.1 and 3.8.

Arrangement of nerve in the moist chamber is illustrated in diagram E. Explanation in text.

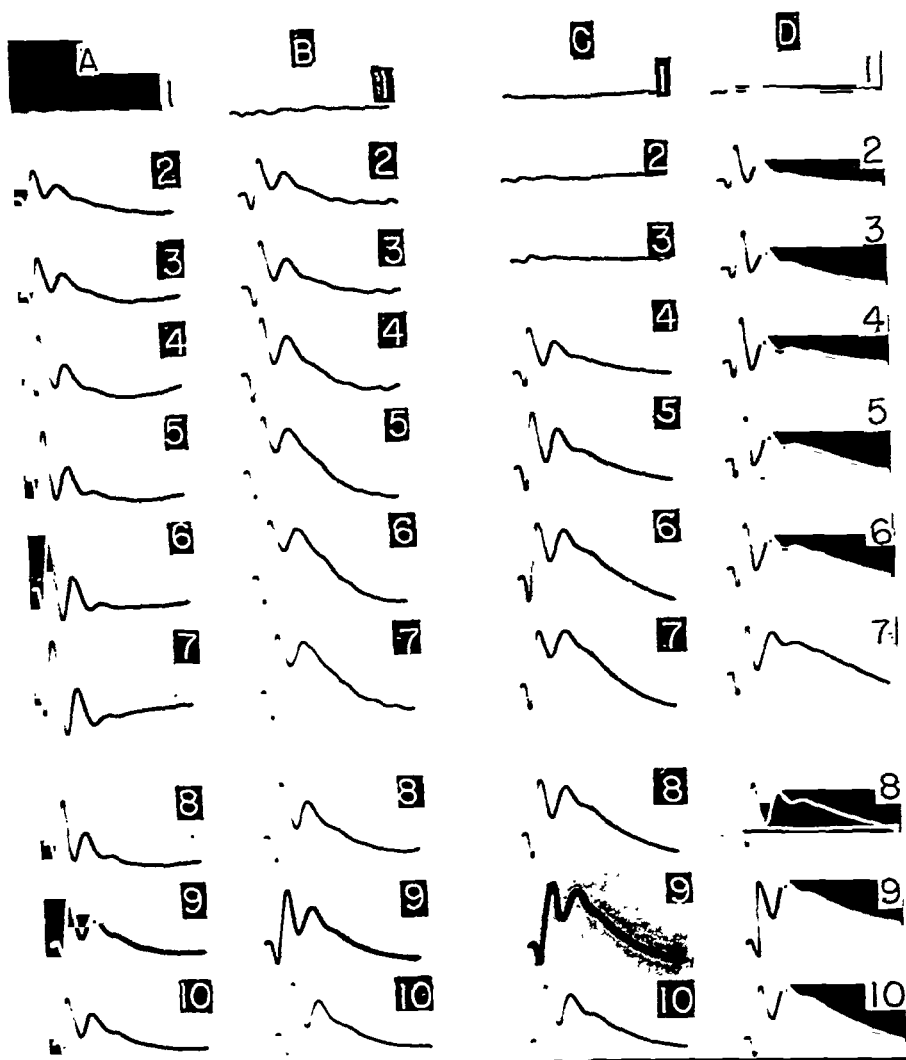


FIG. 4. Action potentials to single stimuli of increasing strengths (1-7) from the sciatic-tibial nerves of control frog 18 (A, B) and experimental frog 17 (C, D). Relative strengths of stimuli for records 1-7 were: 1.0, 1.1, 1.2, 1.4, 1.7, 2.1, and 2.6. The neurograms for single stimuli are also shown for all 4 nerves before (8) and after (10) a ten minute period of repetitive stimulation at 14 shocks per second and at a shock strength of 1.4. The terminal portion of the ten minute period of repetitive stimulation is shown for all nerves as record 9.

#### INTERPRETATION

The results presented lead to the conclusion that the acetylcholine splitting powers of a nerve can be virtually reduced to zero by the *in vivo* administration of DFP, and that this condition can be maintained more than

long enough to test the ability of such a nerve to conduct impulses. Such tests showed that these nerves were able to conduct impulses as well as normal nerves. There appears to be, in the case of peripheral nerve fibers, no relation such as Nachmansohn *et al.* (21, 22) reported in the electric eel, between the cholinesterase concentration and the potential developed. It is doubtful, then, that the block obtained when DFP was locally applied could have been due to its anticholinesterase action. Such an interpretation is hardly in accord with the facts brought out by either the *in vitro* or the *in vivo* series of experiments. No attempt was made to determine the exact cause of block when DFP was locally applied, but a number of factors such as depolarization, electrotonic effects, the electrical properties of the fluid medium about nerve fibers (3, 18, 27) have been considered to be significant in producing such local blocks. It is doubtful, also, if the eserine block which was obtained was the result of an anticholinesterase action, since Cowan (8) soaked nerves for periods up to 3 hours in eserine and obtained no evidence of an effect when the nerves were taken out of the drug and tested oscillographically. This result was confirmed in an *in vivo* experiment by Cantoni and Loewi (6). The production of local blocks may well depend on the immersion or contact of a segment of the nerve with the drug, thus resulting in the flow of electrochemically induced currents which lead to electrotonic-like blocks. A compound like eserine salicylate may be ineffective in the concentrations employed, because it may not have such electrochemical effects. Irrespective of the exact mechanism involved in the block, the conclusion appears inevitable that nerve fibers can conduct in the absence of cholinesterase. Evidence associated with the distribution and concentration of cholinesterase has in the past been called forth in deductions about the role of acetylcholine in the conduction of the nerve impulse. It is this same kind of evidence which is presented here, and which appears to cast serious doubt on the role of acetylcholine as a depolarizer in the process of conduction. This conclusion should not be construed to imply that acetylcholine and cholinesterase play no role in the physiology of peripheral nerves. It may be that nerves without cholinesterase are unable to recover after prolonged activity as quickly as normal nerves, or have abnormal trophic properties or show impairment in other ways. The fact that nerves conduct impulses does not indicate that they are normal in every respect. In fact, the absolute refractory period of frog sciatic nerve has been shown (1) to be prolonged by the application of eserine and prostigmine.

#### SUMMARY AND CONCLUSIONS

1. Local application of eserine or di-isopropyl fluorophosphate (DFP) in Ringer solution to segments of isolated nerves of the cat or bullfrog led to a block of nerve impulses, indicated by the failure to record action potentials in the nerve beyond the region of application.

2. Such a block was not irreversible but was abolished by washing the

exposed segment of the nerve in Ringer solution, or in the case of DFP by merely lifting the nerve out of solution of the drug.

3. Eserine salicylate in the same concentration (0.01–0.02M) had no blocking action when applied locally.

4. The in vivo administration of DFP to bullfrogs produced a reduction in the cholinesterase content of the nerves to a mean value of 2.3 per cent of that from the control nerves. This indicates that the experimental nerves had virtually no acetylcholine-splitting activity. Such nerves, however, were found to conduct impulses equally as well as the control nerves following either single or repetitive shocks at frequencies as high as 43 per second.

5. The conclusion is reached that in nerve fibers there is no parallel relationship between the magnitude of the action (spike) potential and the cholinesterase activity as determined on the nerves after homogenization. It appears that the block which was produced by local application of DFP was not one resulting from the anticholinesterase action of this compound.

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# EFFECT OF DI-ISOPROPYL FLUORPHOSPHATE (DFP) ON ACTION POTENTIAL AND CHOLINE ESTERASE OF NERVE.\*†

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## INTRODUCTION

EXPERIMENTS, on the giant axon and fin nerve of squid have shown that anti-choline-esterases abolish the action potential (1). When the nerve is immersed in a solution of eserine, the action potential is altered and eventually disappears. When the nerve is put back into sea water, the action potential rapidly reappears. Since the inhibition of choline esterase by eserine is easily reversible, these data are consistent with the concept that the release of acetylcholine is an essential link in the changes occurring in the membrane during the passage of the nerve impulse. As a corollary of the same concept, it may be expected that only those inhibitors of choline esterase which pass through the lipid membrane surrounding the axon affect the action potential. Prostigmine, in the same concentration as eserine, namely, about  $10^{-8}$  M, causes the same amount of inhibition of choline esterase in the test tube. However, prostigmine when applied to the axon, does not affect the action potential.

Recently a new inhibitor of choline esterase, di-isopropyl fluorophosphate (DFP) became known, which can inhibit choline esterase irreversibly. In this paper, observations will be described in which the parallel effects of DFP on the nerve action potential and on choline esterase have been studied.

## METHODS

The experimental conditions and methods used for the observations on squid nerves were exactly the same as described previously (1).

Lobster nerves (abdominal chain) were used in the later series of experiments. The action potentials resulting from single electrical stimuli to the freshly prepared, untreated nerve were recorded oscillographically. The nerves, usually still on the electrodes, were then transferred to sea water prepared according to Pantin (3) and containing usually 2 mgs. of DFP per cc. Action potentials were recorded intermittently. After the action potential had disappeared, the nerves were either washed in oxygenated sea water or kept for different additional periods of time in DFP and then washed in sea water. The sea water was changed frequently and a steady stream of oxygen was passed through it in order to accelerate the removal of the DFP. The nerve was then cut into two approximately equal parts.

\* The work described in this paper was carried out under a contract between The Chemical Warfare Service, U. S. Army, and Columbia University.

† In accordance with *Journal* policy, the Editors have respected the authors' preference for treating cholinesterase as two words. The enzyme is the same as that discussed in the previous paper.—Ed.



One half was homogenized and the choline esterase activity was tested directly with the usual manometric technique. The second half was ground as above but added to a highly active solution of choline esterase. In this way, the inhibition due to the possible remainder of DFP in the connective tissues after washing in sea water was determined.

## RESULTS

*A. Squid nerve.\** DFP, tested on the fin nerve of squid, has the same effect on the action potential as was observed with eserine and at about the same concentration. The effect on the action potential is pronounced in 10 minutes when the nerve is kept in a solution of 2 mgs. of DFP per cc. (0.013 M), but as may be seen in Figure 1, the action potential is completely abolished only after 35 minutes. When the nerve is put back into sea water, a partial recovery is observed after 60 minutes. Recovery is nearly complete after 205 minutes in sea water.

In lower concentration of DFP, namely, 1 mg. per cc. (0.0065 M), the effect is produced at a considerably slower rate. Figure 2 shows that even after 90 minutes the abolition of the action potential is incomplete.

It was concluded from these experiments that for relatively short periods and at low temperature, around 20°C, the inactivation of choline esterase by DFP is partly reversible. Unfortunately, the season was too advanced to check the choline esterase activity in squid nerves under the experimental conditions used in order to compare the changes in enzyme activity with the alterations of the action potential.

*B. Lobster nerve.†* Experiments were therefore carried out later with the ventral abdominal nerve cord of the lobster, which provides nerves high in choline esterase activity and with relatively satisfactory action potentials.

*Effect of DFP on action potential.* When the nerve is immersed in a solution of 2 mgs. (0.013 M) of DFP per cc., the action potential of the lobster nerve preparation also disappears within about 30 to 40 minutes. With a concentration of 3 mgs. (0.02 M) of DFP per cc., it takes about 15 minutes until the action potential disappears, as shown in Figure 3. If the nerve preparation is put back into sea water immediately after the abolition of the action potential, the response reappears (Fig. 4, column 1). As with squid nerve, the recovery requires a certain length of time. Nerves kept in DFP for additional periods after abolition of the action potential show less complete recovery after washing with sea water. The longer the nerve is exposed to the action of DFP, the less complete is the recovery. Additional exposure of the nerve to DFP for 90 minutes after the disappearance of the action potential, causes irreversible abolition of the potential. Figure 4 illustrates the degree of reversibility of the action potential if the nerve is washed in sea water immediately after the disappearance of the action potential or kept in the DFP solution for 30, 60, and 90 minutes respectively.

\* These experiments were carried out in the summer of 1945 at Marine Biological Laboratory, Woods Hole, by T.H.B., D.N., and M.A.R.

† These experiments were carried out in the winter of 1946, at the Dept. of Neurology, College of Physicians and Surgeons, Columbia University, by H. G., D.N., M.A.R., and K.S.

*Effect of DFP on choline esterase.* Determinations of choline esterase in these nerves reveal a parallelism between the recovery of the action potential and the reappearance of choline esterase as shown in Figure 4. The less complete the recovery of the action potential, the smaller is the amount of

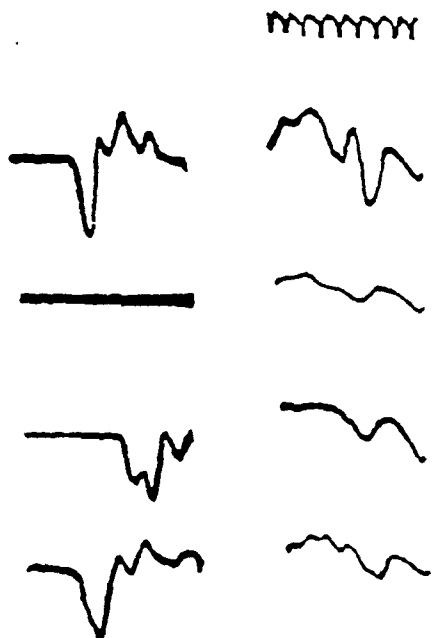


FIG. 1. Effect of DFP on the action potential of the fin nerve of squid. DFP 0.013 M. First two records (from top to bottom): before, and after 35' in DFP. The last two records: recovery after 60' and 215' in sea water.



FIG. 2. Fin nerve of squid. DFP 0.0065 M. From top to bottom: before, and after 8', 17', 38', 98' in DFP. Last record: recovery after 115' in sea water.

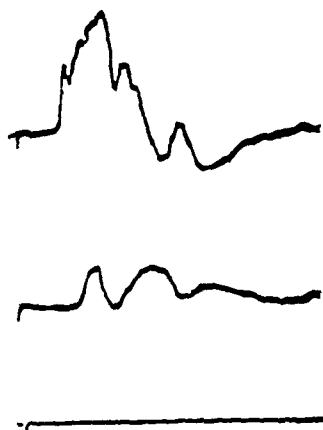


FIG. 3. Lobster nerve (abdominal chain). DFP 0.02 M. From top to bottom: before, and after 10' and 15' exposure.

choline esterase activity. Even after complete and irreversible abolition of the action potential, a small amount of enzyme activity may still be detected.

In case of full reactivation of the action potential after DFP poisoning,

the enzyme activity leads to an output of about 250 cmm.  $\text{CO}_2$  per 100 mgs. per hour. Since the  $\text{CO}_2$  output of an untreated preparation of the abdominal chain of lobster is about 1,300 to 1,500 cmm. per 100 mgs. per hour, the remaining activity is about 15 to 20 per cent of the normal.

*DFP retained after washing nerve.* It may be expected that even after

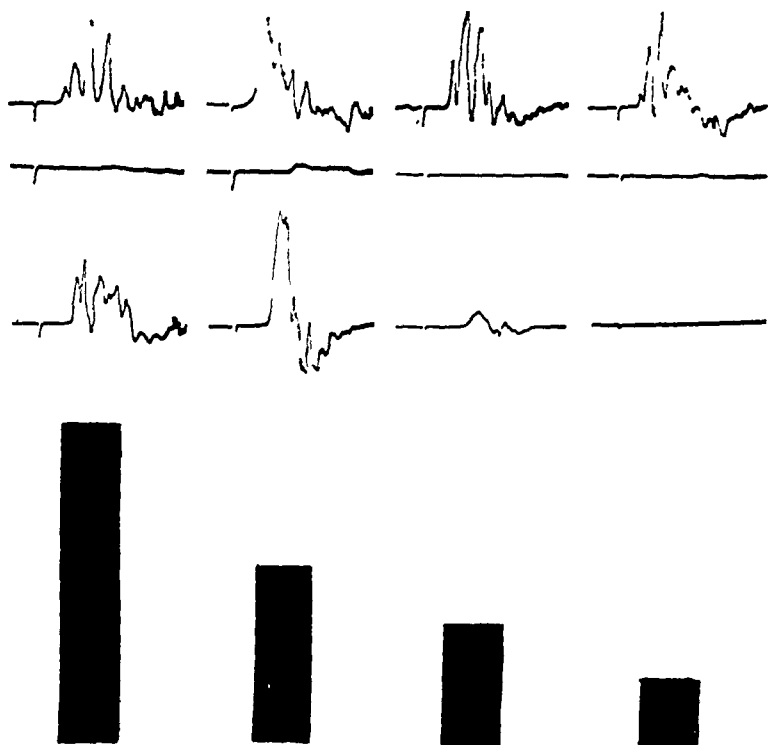


FIG. 4. Reversibility of action potential and reappearance of choline esterase in nerves exposed for varying periods of time to DFP, 0.013 M. The nerve whose action potentials are shown in col. 1 was transferred to sea water immediately after the action potential was abolished and washed for one hour. The nerves of col. 2 to 4 were kept in DFP for 30', 60' and 90' after the action potential had disappeared and then washed in sea water. The top line of each column shows the action potential in the untreated nerves. The second line shows the abolition of the response by DFP. The third line shows the degree of recovery after washing the nerve. The reappearance of choline esterase activity is shown in the vertical bars of the fourth line. The  $\text{CO}_2$  output is 233, 129, 88.5 and 50 cmm. per 100 mgs. per hour.

washing, a small amount of DFP is retained. The  $\text{CO}_2$  output measured directly would therefore not indicate the total esterase activity. The inhibition due to the DFP remaining in the nerve has been measured as described above (see Methods) and varies between 20 and 40 per cent (see Table 1). The percentage inhibition remains constant for a given concentration of DFP independent of the concentration of choline esterase (Table 2). It is therefore

possible to correct for the inhibition of the choline esterase in the nerve due to the remaining DFP.

If nerves which have been exposed to DFP are ground in a highly active choline esterase solution without preliminary washing to remove the DFP, the total enzyme activity of the added solution is abolished. In a typical experiment the activity of the choline esterase solution fell from 1,400 cmm. per hour to 13 cmm. per hour. Therefore the enzyme activity of the nerve cannot be determined without preliminary removal of DFP by washing.

*Table 1. Inhibition of the activity of a choline esterase solution added to nerves kept in DFP and homogenized after washing in sea water for one hour. CO<sub>2</sub> output of the choline esterase solution was 1460 cmm. per hour. The nerve of Experiment 5 was kept in DFP until disappearance of the action potential and then washed for three hours.*

Exp.	CO <sub>2</sub> output cmm./ hr.	Inhibition in per cent	Nerve of Fig. 4 column
1	876	40.0	1
2	781	46.5	2
3	1,164	31.0	3
4	1,195	20.3	4
5	909	37.7	

*Table 2. Inhibition of varying concentrations of choline esterase by 0.4 µg. of DFP per cc. [Two experiments.]*

Control	CO <sub>2</sub> output per hour with DFP	Inhibition per cent
534	346	35
240	165	31
111	70	37
565	316	44
280	160	43
142	89	37

On the other hand, washing of the nerve in sea water for periods longer than one hour, does not seem to improve DFP removal as may be seen from Experiment 5, Table 1, where the nerve had been washed for three hours.

DFP is quite lipid soluble. In order to remove the compound more completely from nerve lipid tissues, washing the tissue with propylene glycol has been suggested.\* However, washing with propylene glycol leads to a considerable loss of choline esterase activity. In two nerves not treated with DFP, the choline esterase activity fell after twenty washings from about 1,400 cmm. CO<sub>2</sub> per 100 mgs. per hour to 576 and 597 cmm. CO<sub>2</sub> respectively. In two nerves soaked in DFP for 40 minutes and then divided into approximately equal parts, the halves of each nerve which were washed in

\* Gilman, *et al.* Symposium on the physico-chemical mechanism of nerve activity' held at the New York Academy of Sciences on February 8 and 9, 1946.

sea water gave an output of  $\text{CO}_2$  of 158 and 124 cmm. per 100 mgs. After twenty washings in pyropylene glycol, the activity obtained in the other two halves was 39 and 21 cmm.  $\text{CO}_2$  respectively.

C. *Reversibility of inhibition of choline esterase by DFP in vitro.* Irreversibility of enzyme inhibition is the function of a number of variables. Two essential factors are the temperature at which the process occurs and the time required. The experiments described above indicate that the irreversible inhibition by DFP of the total amount of choline esterase present in nerves progresses rather slowly at room temperature, and may be partly reversed within one to two hours. It appeared desirable to bring additional

Table 3. *Reversibility of choline esterase inhibition by DFP in vitro, tested by dilution effect. Temperature 9°C. The choline esterase solution used liberates 790 cmm.  $\text{CO}_2$  per hour. The two left columns show the inhibition of the choline esterase by 0.1  $\mu\text{g}$ . of DFP per cc. at different time intervals after mixing. The right columns show the activity found after exposure to 0.5  $\mu\text{g}$ . of DFP per cc. for varying periods of time and subsequent dilution. The last column shows per cent inhibition of the values of column 4 as compared with those of column 2.*

Diluted solution 0.1 $\mu\text{g}$ . DFP per cc		Diluted after exposure to 0.5 $\mu\text{g}$ . DFP per cc		Per cent inhibition
time min.	$\text{CO}_2$ output cmm./hr.	time of dil. min.	$\text{CO}_2$ output cmm./hr.	
initial	520	2	493	5
		6	498	4
		18	400	23
54	494	54	280	43
162	390	162	41	89
320	283	320	0	100

evidence for the possibility of a partial reversal of the inhibition of choline esterase by DFP *in vitro*.

Table 3 shows an experiment in which DFP was added to a choline esterase solution prepared from electric tissue. The temperature used throughout the experiment was 9°C. One cc. of the enzyme solution used liberates 790 cmm.  $\text{CO}_2$  per hour if no DFP is added. DFP in a concentration of 0.5  $\mu\text{g}$ . per cc. inhibits 75 per cent of the activity immediately and after 30 minutes incubation 94 per cent of the activity is lost. If, however, the concentration of DFP is 0.1  $\mu\text{g}$ . per cc., the  $\text{CO}$  output is 520 cmm. as compared with 790 initial, *i.e.*, the inhibition is only 34 per cent. The activity in presence of this concentration of DFP decreases only slowly (left columns of Table 3). After 320 minutes, more than 50 per cent of the initial activity is still present.

At the right side of Table 3 is shown the activity when the enzyme solution is first exposed to 0.5  $\mu\text{g}$ . of DFP per cc. for varying periods of time and then diluted. If the enzyme is reversibly inhibited, a dilution after exposure to a higher concentration of the inhibitor leads to a partial or complete reactivation. On the other hand, in case of irreversible destruction, no such reactivation is possible by dilution. The figures show that reactivation does occur. After 54 minutes' exposure to the high concentration of DFP, a con-

siderable part of the enzyme has been reactivated by dilution. The activity is only 43 per cent smaller than that of the enzyme exposed from the beginning to the low concentration of DFP. Even after 162 minutes, a small reactivation still occurs. The data show that even *in vitro*, choline esterase inhibition by DFP is, at low temperature, partly reversible for a considerable length of time.

### DISCUSSION

The abolition of the action potential by DFP poisoning is reversible, but only for a period of time. If the nerve is kept in the solution of DFP after abolition of the potential, recovery of the action potential becomes progressively less. If under these conditions, the reversibility of the action potential is compared to the amount of choline esterase which reappears, a striking parallelism is obtained. It is, however, desirable to test this relation further on other nerve preparations and such experiments will be presented in a subsequent paper.

The *in vitro* experiments give additional evidence that the irreversible inhibition of choline esterase by DFP is a slow process at low temperature.

There is no reason to doubt that DFP penetrates the lipid membrane into the interior of the axon. Previous observations as mentioned above have shown that only those compounds affect the action potential which pass through the lipid membrane. Eserine and DFP have about the same anti-choline-esterase effect and since they abolish the action potential at about the same concentration in the same period of time, the effect of DFP is consistent with the view that the abolition of the action potential occurs by means of inhibition of the choline esterase.

Recently, observations on bull frogs were reported in which it was found that following injection of DFP, the action potential of the sciatic nerve may persist in the apparent absence of choline esterase (2).<sup>\*</sup> The bull frog sciatic nerve contains extremely small amounts of choline esterase. 100 mgs. of nerve (wet weight) liberates 40 to 50 cmm. CO<sub>2</sub> per hour, the activity therefore being smaller than in most myelinated nerves. This low activity may be correlated with the relatively small amount of active surface and the large amount of inactive tissue in the bull frog sciatic nerve.

The figures for lobster nerve given above indicate that the enzyme is present in about five times excess since about 80 per cent may be removed while the action potential is unaffected. Such an excess of enzyme above the minimum required is quite frequent. Even if in the bull frog sciatic nerve the excess of enzyme is smaller, when part of the activity disappears, the measurement of the CO<sub>2</sub> output falls into a range where precise determination becomes difficult.

<sup>\*</sup> The data were also presented by Gilman at the symposium on the physico-chemical mechanism of nerve activity, held at the New York Academy of Sciences on February 8 and 9, 1946.

The retention of  $\text{CO}_2$  by the protein also becomes an important factor. Moreover, excess of DFP which is retained even in the thin lipid membranes of the lobster nerve is sufficient to inhibit 20 to 40 per cent of the remaining esterase activity in spite of prolonged washing of the tissue. It therefore appears probable that at least this amount, if not more, is retained in the relatively greater amount of inactive tissue present in the bull frog sciatic nerve. When this nerve is then ground, the retained DFP comes in contact with the choline esterase and may destroy a considerable fraction of of the choline esterase still active in the intact nerve.

### SUMMARY

1. DFP, like other anti-choline esterases, abolishes the action potential of the fin nerve of squid. The same effect at the same concentration and in the same period of time is observed on the abdominal chain of lobster.

2. When the nerves are washed in sea water immediately after the disappearance of the action potential, the response reappears completely or nearly completely.

3. If, however, the nerve is kept in DFP for various additional periods of time, reversibility becomes increasingly incomplete and eventually the action potential is irreversibly abolished.

4. The degree of reversibility of the action potential is strikingly parallel to the amount of choline esterase which reappears in the nerve preparation of the lobster.

5. The experiments indicate that choline esterase inhibition by DFP in nerves of cold-blooded animals is partly reversible for a certain period of time.

6. This is confirmed by observations on *in vitro* inhibition of choline esterase solution.

7. The observations are consistent with the concept that the release and rapid removal of acetylcholine is an essential event during conduction.

We are obliged to Miss Helen Schneemann for help in some choline esterase determinations.

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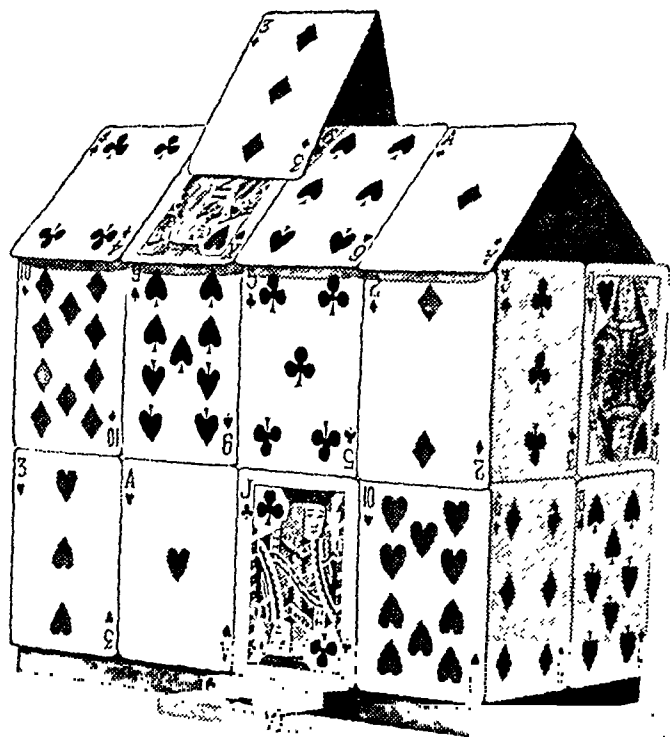
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THE RELATION of agonist to antagonist in voluntary movements is controversial. Some authors such as Watkins (17) are inclined to consider simultaneous activity of flexors and extensors ("co-contraction") as a pathological symptom frequently seen in poliomyelitis, whereas others emphasize its occurrence in normal individuals (4, 6, 7, 16). As a physiological foundation for the understanding of the mechanisms of muscular coordination under conditions of voluntary innervation, a detailed knowledge of the function of muscles following electrical stimulation of the motor cortex is of value. Hering and Sherrington (9) concluded, on the basis of visual observation and palpation of muscles, that muscle activity following cortical stimulation occurs in accordance with the principle of reciprocal innervation. Under other conditions Graham Brown and Sherrington (2) found that flexor and extensor muscles may also be activated simultaneously on stimulation of the motor cortex. Loewenthal and Horsley (12) and Tilney and Pike (15) recorded under similar conditions simultaneous activity of biceps and triceps. Hering (8) criticized the experiments of the former on the grounds that simultaneous contraction of biceps and triceps leading to fixation of the elbow joint is well known to occur as a result of cortical stimulation or voluntary innervation. Under these conditions biceps and triceps must be considered to be synergists, although the same muscles may be antagonists during flexion and extension. Since attachment of the tendons to the recording device precluded disclosure of the type of movement which resulted from cortical stimulation the interpretation of the simultaneous activity of biceps and triceps is uncertain (8). If it were possible to demonstrate that with the performance of a flexor or extensor movement both biceps and triceps would contract simultaneously, the existence of co-contraction under physiological conditions would be proven. In view of the fact that electromyographic records are a sensitive indicator of muscle activity and since the insertion of electrodes into the muscles hardly interferes with the normal performance of movements, a series of experiments was undertaken in which muscular coordination was studied under conditions of cortical stimulation by means of electromyograms (E.M.G.'s.). That the E.M.G.'s resulting from stimulation of the motor cortex signify muscle contractions is well established (3).

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## METHOD

This investigation is based on experiments performed on 32 cats and 3 monkeys (macaque) under light narcosis (.45 cc. dial-urethane/kg. intraperitoneally). The head was held rigidly in the Horsley-Clarke apparatus; one hemisphere was exposed and stimulated with condenser discharges through bipolar silver electrodes with an interelectrode distance of 1-2 mm. Stimuli of threshold intensity or slightly (1-3V) stronger were applied for 10 seconds. (For further details cf. 13.) Fine copper wires, the intramuscular portion of which was bared, were inserted into the muscles and led to the input of a push-pull amplifier. The potentials were recorded with an Offner inkwriter.

## RESULTS

Before the more complex results concerning muscular coordination are discussed it is desirable to illustrate the effect on a single muscle of cortical

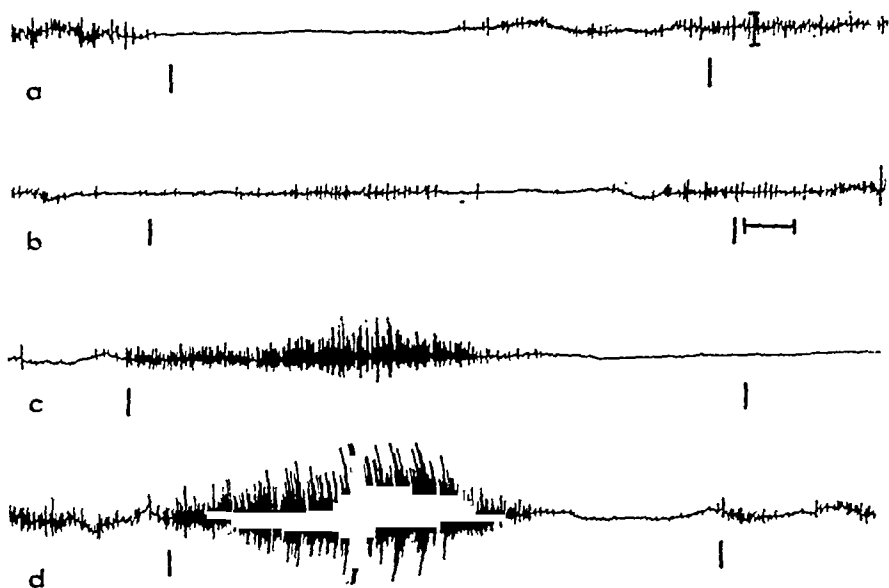


FIG. 1. E.M.G. of hamstrings on stimulation of motor area of the cat with 4.0 (a), 5.3 (b), 6.3 (c) and 7 Volts (d). Duration of stimulation 10"; frequency 45/sec. All graphs are to read from right to left.

excitation with increasing intensity of stimulation. In order to show inhibitory as well as excitatory effects some pre-stimulatory tonic activity is necessary. In the example illustrated in Figure 1 it was present in the hamstrings of a cat and was inhibited when the cortex was stimulated with an intensity of 4 volts. The record shows that the inhibition gradually increased so that the tone was abolished after an interval of about six seconds. Following the cessation of the stimulation the original tone reappeared to an increased degree. This is considered to be the expression of a rebound similar to that frequently seen in decerebrate preparations after an inhibition of extensor muscles.

At a slightly greater intensity of stimulation (5.3V) the tone was in-

hibited during the early and late part of the stimulation period while some activity was noted between these two periods of inhibition. The interpretation of this activity is suggested by the study of records c and d of Figure 1 in which, after an interval of a similar duration, distinct electromyographic potentials appear. Comparison of records b, c and d of Figure 1 permits one to state that after a long "latent period" (summation time), which decreases with increasing intensity of stimulation, there appears a period of activity which is delimited by zones of inhibition provided that a tonic activity is present. Such pre- and post-stimulatory periods of inhibition are seen in records b and d. The difference between the two records is mainly one in degree. The excitation is greater in intensity and duration in record d than in record b; conversely the inhibition seems to be more marked in record b than in record d. This is shown particularly by the fact that in record d the post-stimulatory period of inhibition is not well demonstrated. These findings are in line with other observations in which it was noted that with increasing degree of stimulation the inhibitory zones which precede and follow increased muscle activity become gradually shorter and often finally disappear.

When the electromyograms of a pair of antagonistic muscles such as the quadriceps and semitendinosus, or the biceps and triceps, were recorded and the effect of cortical stimulation was studied, the various phases (inhibition, excitation) illustrated in Figure 1 can be evaluated with respect to the agonist-antagonist relationship. Tonic activity required to demonstrate the full range of effects of cortical stimulation was found most frequently in the triceps or hamstring muscles although some animals showed some tone in the biceps brachialis also. Tonus was observed in 9 cats and manifested itself either in tremor-like movements or in action potentials not accompanied by gross signs of muscular activity.

Inhibition as indicated by a diminution or disappearance of muscle action potentials as a result of cortical stimulation was obtained in general from the whole excitable motor cortex. In only 2 of the 9 cats in which inhibition of tone was studied was it found that parts of the excitatory motor cortex failed to inhibit the extensor tone present in the quadriceps femoris. There were quantitative differences in the inhibition of the fore- and hindlimb muscles on cortical stimulation. Inhibition of the tonus of the forelimb muscles was more prolonged and resulted from a wider range of intensities of stimulation than were found effective to produce inhibition in the hindlimb.

Electromyograms of flexors and extensors of the knee show inhibition under the influence of cortical stimulation at low intensity (Fig. 2a), and excitation at higher intensity (Fig. 2b). The tone in the extensor muscle is progressively inhibited after a latent period of about 2 seconds. The minimal activity appearing in the flexor just before the onset of stimulation is also eliminated, possibly indicating some inhibition. Following cessation of stimulation there is a period of increased activity in both muscles (rebound).

As Graham Brown and Sherrington (2) pointed out, this rebound may be

seen in flexors as well as extensors following cortical stimulation. However, the relation of inhibition to post-inhibitory rebound under conditions of cortical stimulation was less clearly established in the experiments of these authors since in both illustrations (their Figs. 21 and 22) the rebound was seen in the myogram of the antagonist although this muscle failed to show inhibition during cortical stimulation.

As the intensity of stimulation increases (cf. Fig. 2b) a flexion of the

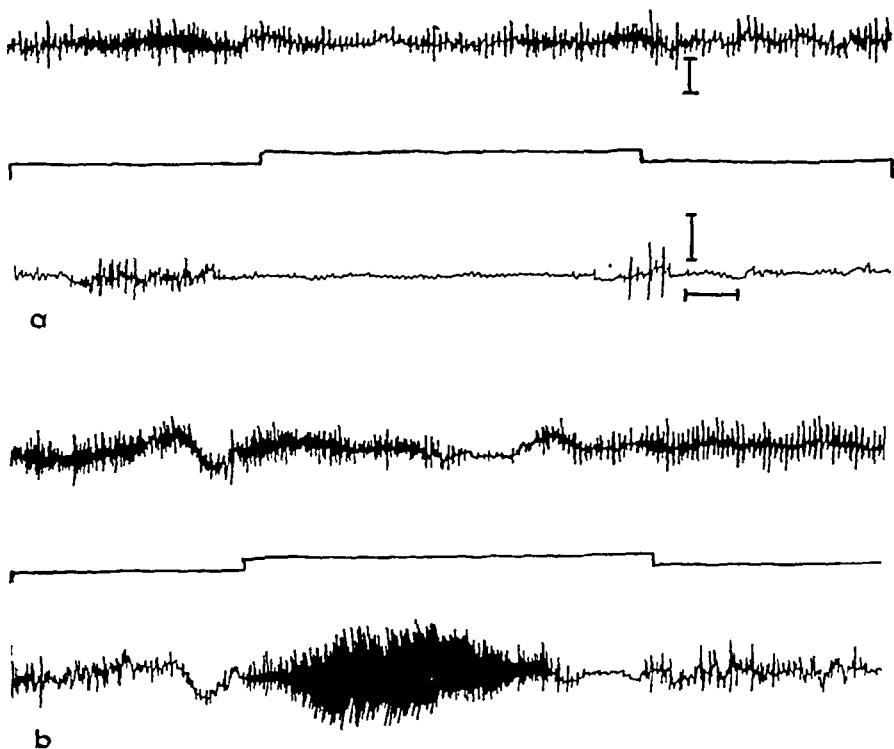


FIG. 2. E.M.G. of quadriceps femoris (upper line) and semitendinosus-semimembranosus (lower line) on stimulation of motor cortex (cat) at a frequency of 85/sec. for 10 seconds.

Figure 2a: Intensity 3.6V; no visible movement

Figure 2b: Intensity 7.0V; flexion of knee

hindleg is seen which is accompanied by characteristic changes in the E.M.G., indicating excitatory as well as inhibitory phenomena. About 1/5 second following the onset of stimulation, there is an abrupt inhibition of the tone of the flexor muscle while the extensor tone is not significantly altered. This period of flexor inhibition is followed by one of flexor excitation, the beginning of which is associated with inhibition of the extensor tone. However, the latter period is only brief and is followed by a gradually increasing activity which is clearly distinguished from the pre-stimulatory tonic activity because of the discontinuous character of the latter. It is worthy of

note that for a certain interval the activity increases in the flexor as well as in the extensor muscle, although the extensor muscle shows its maximal activity at the end of the period of stimulation while the activity of the flexor muscle has already declined at this time. The cessation of stimulation is followed by a brief period of diminished amplitude of the action potentials. Hereafter a period of increased activity (rebound) is noted in both muscles.

The experiment of Figure 2 demonstrates, aside from the data related to

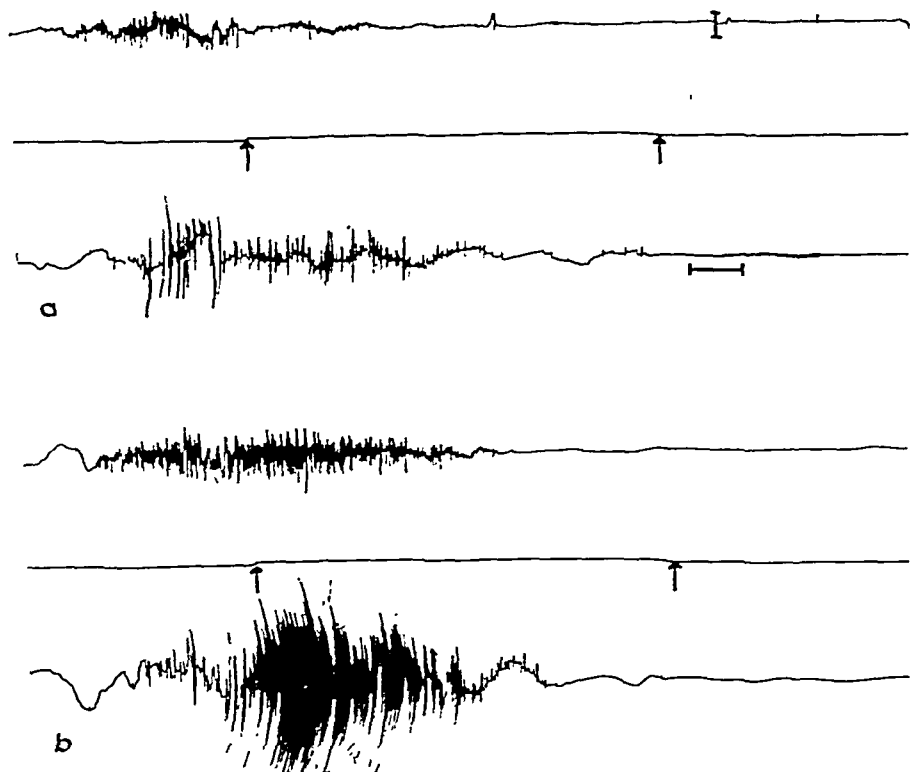


FIG. 3. E.M.G. of quadriceps (upper line) and semitendinosus (lower line) on stimulation of motor cortex (cat) at a frequency of 85/sec. for 10 seconds.

Figure 3a: Intensity 4.5V; slight flexion of knee

Figure 4a: Intensity 5.3V; strong flexion of knee

cortically induced inhibition and rebound, the fact that a flexion of moderate intensity may be accompanied by simultaneous activity of both extensor and flexor muscles. This co-innervation is apparently in sharp contrast to the principle of reciprocal innervation. However, the observation that at the onset of the activity of the agonist (flexor) the tone of the extensor is inhibited shows the close relation between reciprocal innervation and co-innervation under conditions of cortical stimulation. Figure 2b illustrates indeed that with increasing duration of stimulation reciprocal innervation

passes into co-innervation which accompanies co-contraction.

This co-innervation was the most frequent response of antagonistic muscles to any but threshold stimulation of the cortex. It was observed in pairs of muscles which were antagonists with respect to the movement of the knee and elbow. It occurred in all but four of the 32 cats studied and was associated with slight and moderate movements of flexion or extension.

Whereas Figure 2 illustrates the temporal transition from reciprocal in-

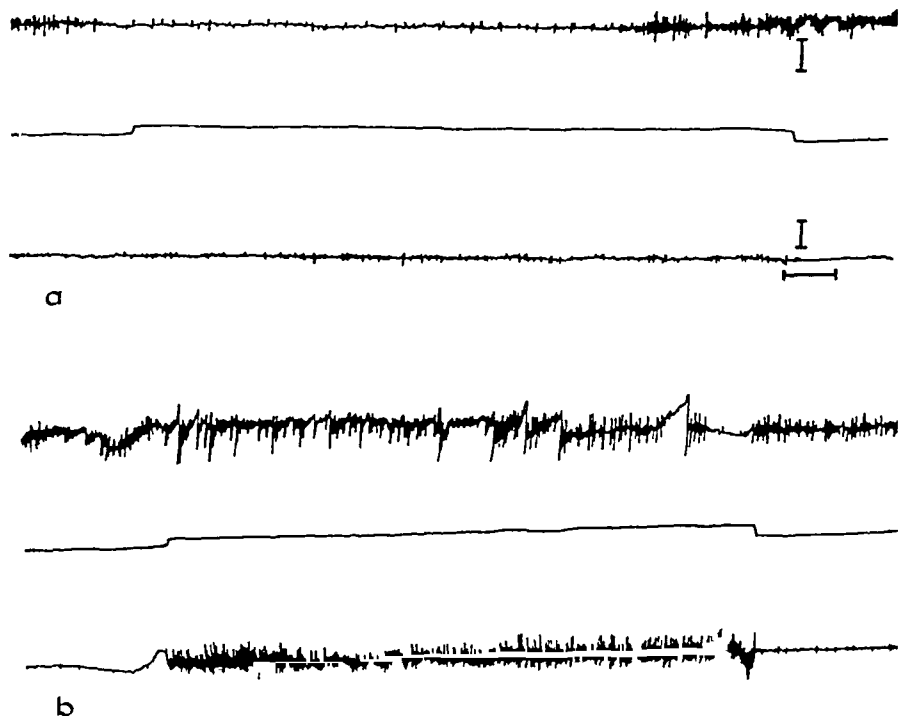


FIG. 4. E.M.G. of triceps (upper line) and biceps (lower line) on stimulation of motor cortex (cat) at a frequency of 45/sec. for 10 seconds.

Figure 4a: Intensity 2.5V; no visible movement

Figure 4b: Intensity 3.6V; slight extension of elbow

nervation to co-innervation during the period of cortical stimulation, Figure 3 may serve to demonstrate the relation of co-innervation to the intensity of cortical stimulation. Figure 3a shows only a trace of activity in the extensor muscles toward the end of the stimulation period whereas distinct action potentials appear in the record of the agonist (flexor). After the end of the stimulation there is some after-discharge in the flexor muscle and a distinct rebound in the extensor. The appearance of this "rebound" after little or no response during the period of stimulation is analogous to the myographic observations of Graham Brown and Sherrington (2) mentioned above. While stimulation at 4.5V leads only to a slight movement, stimulation at a slightly

higher intensity causes a distinct flexion of the knee which is accompanied by simultaneous activity of both flexor and extensor muscles. Attention is called to the fact that a muscle which under conditions of stronger stimulation is called into action may, on weaker stimulation, show activity only in the form of an after-discharge. This fact observed by Murphy and Gellhorn (13) is confirmed here on the basis of electromyographic records.

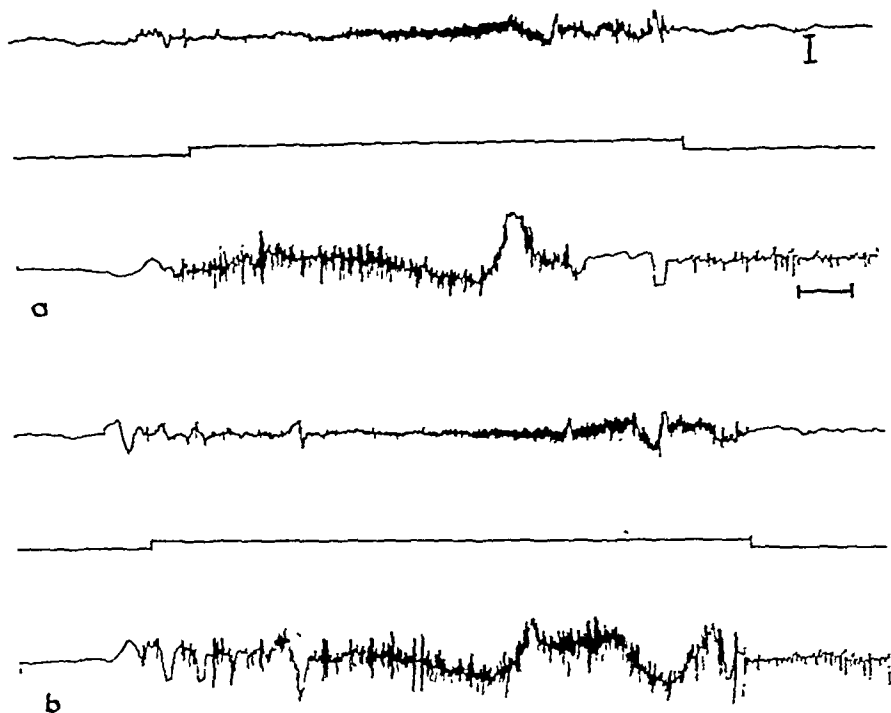


FIG. 5. E.M.G. of quadriceps (upper line) and semitendinosus (lower line) on stimulation of motor cortex (cat) at a frequency of 55/sec. for 10 seconds.

Figure 5a: Intensity 5.3V; slight extension of knee

Figure 5b: Intensity 7.0V; slight extension of knee

Figure 4 illustrates the relation existing between inhibition, reciprocal innervation and co-innervation for a cortically induced extensor movement. At a very low intensity (2.5V) the extensor tone is greatly reduced (inhibition) while at the same time a trace of activity appears in the flexor muscle. No movement is visible under these conditions. When the intensity is increased to 3.6V a slight extension movement is noted. The E.M.G. reveals definite co-innervation of extensor and flexor muscles. However, here again a temporary phase of reciprocal innervation is noted at the beginning of the stimulation period when excitation of the flexor is temporarily associated with inhibition of the extensor tone.

Figure 5 shows phenomena closely related to those seen in Figure 4. The



records demonstrate the effects of stimulation of a focus in the motor cortex at 5.3 and 7V respectively. The movements in both instances consist of a slight extension which is accompanied by co-innervation of flexor and extensor muscles. This phenomenon is likewise present at a still lower intensity (3.6V). As in the experiments illustrated in Figures 2b and 4b, the co-innervation is preceded by a phase of reciprocal innervation at the beginning of the stimulation period when increased action potentials in the extensor muscle are accompanied by an inhibition of the flexor tone. It is of interest to note that in the experiment shown in Figure 5a the inhibitory phase occurred in the flexor muscle at the beginning of an extension movement whereas it appeared briefly in the extensor record of Figure 4b although the

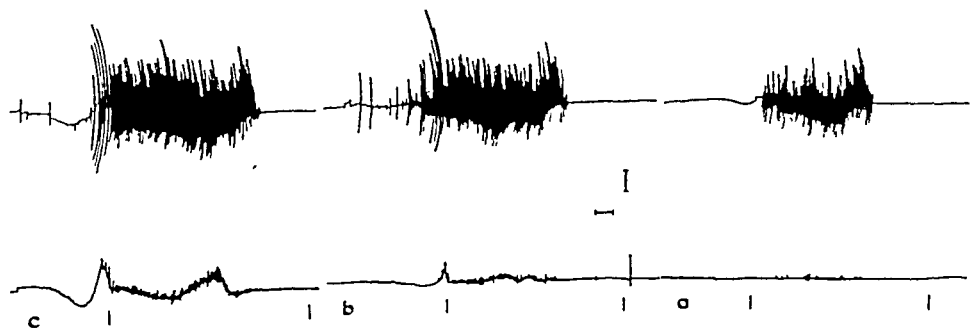


FIG. 6. Stimulation of motor cortex in the macaque (Dial 0.5 cc./kg. i.p.). Condenser discharges, frequency 80/sec. Voltage: 4.0, 4.8 and 5.5 in Figure 6a, b, c respectively.

Upper line: biceps; lower line: triceps

Response in Figure 6 a to c: slight to moderate flexion

movement was likewise that of extension. The E.M.G. record suggests that in the experiment of Figure 4b a very brief flexor activity showing reciprocal innervation precedes the extensor movement which is characterized by co-innervation, whereas in Figure 5a a brief extensor phase with reciprocal innervation precedes the extensor movement that involves simultaneous activation of flexor and extensor muscles. These differences are, however, not noticeable to the naked eye.

The close relation of inhibition and excitation described in this illustration was found commonly in those animals that manifested tonus. In animals in which there was tonus of the agonist muscle, there was the sequence of inhibition and excitation illustrated in Figures 1b and d and 2b; whereas in instances in which the tonus was found in the antagonist muscle, the inhibition of this tonus was often coincident with an active response in the agonist, giving the classical picture of reciprocal innervation. With a longer duration or greater intensity of stimulation, this relation was replaced by that of co-innervation. In those less common instances in which tonus was found in both agonist and antagonist the sequence inhibition—excitation was found in each muscle. The period of inhibition was in most instances not simultaneous in the two muscles but appeared first in the agonist.

It should be emphasized that co-innervation frequently appears under conditions of stimulation at or near threshold without a preceding phase of reciprocal innervation. This is true for the majority of our experiments on cats and monkeys in which muscle tone is absent. If in such preparation the effect of increasing intensities of stimulation on the

E.M.G. is investigated it is found that the lowest effective stimulus excites the agonist only and that with increasing cortical stimulation activity appears also in the antagonist during the period of stimulation.

Figure 6 shows that at an intensity of 4.0V biceps contraction occurs without accompanying activity in the triceps. At 4.8V a slight co-innervation of the triceps is seen while the activity of the biceps increases in intensity. A further increase in activity of biceps and triceps is shown in Figure 6c when the cortex was stimulated with 5.5V. Whereas in this case the movement consisted in slight and moderate flexion, similar E.M.G.'s were obtained in other experiments in which, during the phases corresponding to Figures 6a, b and c, no movement, a barely visible supination and a slight flexion plus supination respectively appeared. It is noted that co-innervation frequently accompanies relatively slight degrees of movement and that increasing innervation of the antagonist coincides with the phase of increasing activity of the agonist. This parallelism in the E.M.G. of biceps and triceps frequently pertains to the E.M.G.'s of the after-discharge.

### DISCUSSION

The fundamental unreliability of palpation as a criterion of the state of contraction has been adequately shown by Tilney and Pike (15). In the light of these findings not too great weight can be attached to the claim of Hering (8) and Hering and Sherrington (7), based on palpation and visual observation, that reciprocal innervation is the typical result of electrical cortical stimulation. The recording of myograms through proper fixation of the leg and attachment of the tendons to a writing lever gives clear evidence of the state of contraction of the muscle, but does not permit one to judge as to whether, under the conditions of stimulation, movement or fixation has taken place (8). Consequently, observations obtained under these conditions may not be decisive for the solution of the question as to the relation of agonist to antagonist in cortically induced movements. Nevertheless, it is of interest to mention that Graham Brown and Sherrington (2) showed reciprocal innervation as well as co-contraction as a result of cortical stimulation, whereas Loewenthal and Horsley (12) and Tilney and Pike (15) observed co-contraction only.

Since in view of the extensive and successful use of the electromyographic method in the last two decades the objections raised earlier by Fulton (5) are no longer valid and the study of action potentials in the muscles of the intact extremity, together with the observation of the movement performed, gives a satisfactory basis for the study of muscular coordination. If, as was frequently the case in our experiments, the extensors and/or flexors showed a distinct tonic activity as revealed by potentials of the muscles which were apparently resting, the influence of cortical stimulation on muscle activity may be fully explored with the inclusion of processes of inhibition. The experiments show under these conditions that with increasing duration or intensity of stimulation the following sequence is found: (i) inhibition of flexors and/or extensors, (ii) reciprocal innervation, (iii) co-innervation of agonist and antagonist.

As was mentioned earlier, the transition from (i) to (iii) may be accomplished by slight increases in intensity, the range varying between a fraction of a volt in some sensitive preparations to that of 1-3 volts in others.

That this sequence may occur in reversed order is suggested by the appearance of temporary phases of inhibition in agonist and/or antagonist at the end of the period of stimulation.

As an interpretation of these findings it is suggested that the various effects of stimulation of the motor cortex described in this paper are directly related to the number of discharging neurons. Increased duration of stimulation has been found to have the same effect as increased intensity of cortical stimulation. As far as the individual cortical neuron is concerned, the effect of increased duration of stimulation at a given intensity consists in increasing the discharge rate from the Betz cells (1). In addition, it is likely, although not yet proven, that under these conditions more cortical neurons are activated (13). This increased rate of discharge as well as the enlarged number of excited cortical neurons must in turn result in a proportionate rise in the number of excited motor horn cells. However, the processes resulting from increased cortical stimulation and facilitation have important extra-cortical components. First may be mentioned the internuncial cells at the spinal level which will be activated to an increasing degree under these conditions. Secondly, afferent proprioceptive impulses may likewise contribute to the increase in response since pyramidal stimulation (11) as well as voluntary innervation (10) increases reflexes based on proprioceptive impulses. Hence, it may be said that as the number of activated motor neurons increases the innervation of the muscles passes from inhibition† to reciprocal innervation, and finally to co-innervation. This interpretation may be applied to the inhibitory phase seen at the beginning of the stimulation period and immediately following it (cf. Fig. 2b). As the number of active neurons increases under the conditions of facilitation, the muscle passes from inhibition into excitation as indicated by the electromyogram. The post-stimulatory period of inhibition may be explained on the same basis since it seems probable that with cessation of stimulation some of the previously discharging neurons continue to discharge while others show no after-discharge. Consequently, a much smaller and continually decreasing number of neurons is active in the post-stimulatory period than was active at the end of the period of stimulation, and this discharge of a small number of neurons is again accompanied by inhibition in line with the argument presented above.

If it is true that excitation of an intermediate number of neurons calls forth a movement in the form of reciprocal innervation, it might be suggested that the activation of additional cortical neurons induces co-innervation and, consequently, co-contraction by mechanisms similar to those involved in the double reciprocal innervation of Sherrington (14). Although a definite stand on this question is postponed until our material on innervation patterns is presented, it may be mentioned that the "co-contraction" due to double

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† That additional factors are involved in the causation of excitation and inhibition need not be emphasized.

reciprocal innervation and the co-innervation most frequently observed in this investigation (cf. Figs. 3b and 6c) differ fundamentally. In the former, opposite changes in activity of agonist and antagonist precede the phase of simultaneous contraction, whereas in co-innervation parallel changes in the activity of flexor and extensor muscles occur. It is for these reasons that co-innervation and consequently co-contraction, *i.e.*, the simultaneous contraction of flexor and extensor muscles during flexion or extension, are considered to be a phenomenon *sui generis*.†

### SUMMARY

Experiments were performed on the effect of stimulation of the motor cortex of cats and monkeys on muscular coordination by means of electromyograms of flexor and extensor muscles. It was found that the effects depend on duration and intensity of stimulation as follows:

1. Very weak stimulation of the motor cortex causes relaxation of flexor and/or extensor muscles if a pre-stimulatory tonic activity is demonstrable in the electromyograms. Such cortically induced inhibition is frequently followed by a post-stimulatory rebound.

2. With slightly increased intensity which calls forth a movement (flexion or extension), reciprocal innervation of agonist and antagonist may be observed whereas somewhat stronger stimuli cause simultaneous activity in flexor and extensor muscles, *i.e.*, co-innervation, and consequently co-contraction, during flexion or extension.

3. Variations in the duration of the period of stimulation exert effects similar to those seen in experiments involving different intensities. Whereas reciprocal innervation appears during the early phase of stimulation it is later followed by co-innervation while the stimulation continues.

4. Even the agonist may show a transition from inhibition to excitation during stimulation at constant frequency and intensity.

5. It is shown that an inhibitory period frequently occurs on cessation of stimulation.

6. The phenomena described under 1-5 are interpreted as indicating that with increasing number of discharging neurons the peripheral effect on individual muscles changes from inhibition to excitation, and in the case of an antagonistic pair of muscles, from inhibition to reciprocal innervation, and finally to co-innervation. Under conditions permitting facilitation the predominant type of coordination of fore- and hindleg muscles in flexor and extensor movements of moderate intensity is that of co-innervation.

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# CENTRIFUGAL DETERIORATION OF ASPHYXIATED PERIPHERAL NERVE\*

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IT IS GENERALLY assumed that when a non-circulated nerve dies, its ability to function is lost either simultaneously or randomly throughout its length. We are not aware that these assumptions ever have been specifically tested. We had reason to believe that neither is correct, and to demonstrate further that which had been suggested by incidental observation, the present experiments were performed. There was found to be a proximo-distal gradient of functional deterioration along the nerve after asphyxial death of the animal, which must be a manifestation of a gradient along the intact nerve during life.

## METHODS

Exposed nerves were used, and their functional ability was determined by stimulating the fibers electrically and observing either the contraction of a muscle or the nerve action potential. After control observations were recorded with the nerve receiving blood, the trachea was clamped and the functional deterioration of the nerve was followed by continuing the observations. The cats used weighed between 2.5 and 3.5 kg. Anesthesia was induced by administration of 50–60 mg. of chloralose per kg. body weight. The motor fibers examined were those emerging from the spinal cord in L6, L7 and S1 ventral roots, traversing the lumbosacral plexus and passing peripherally in the medial popliteal (tibial) nerve. These nerves were surgically exposed and dissected free of surrounding tissue from the spinal cord to the popliteal fossa as completely as conservation of blood supply would allow in individual cases.

When muscle contraction was used as an indicator of nerve function, the gastrocnemius was chosen, and often nerve branches other than those to the muscle were cut. Ventral rami and the lumbosacral cord corresponding to unused ventral roots were severed central to their entrance into the trunk carrying the fibers of the chosen ventral root or roots. Nerve length from spinal cord to muscle was about 165 mm. A series of stimulating electrodes was placed at intervals along the nerve (=root+peripheral trunk) and used in conjunction with an indifferent anal electrode. Usually two stimulating electrodes were placed on the roots and four on the trunk. An alternative was to use a wire loop electrode which could be moved along the nerve. In this case and also when two fixed electrodes were used on the root fibers, only one root, either L7 or S1, was used. By means of these stimulating electrodes the electrical threshold of the motor fibers at several points in their length was determined. The electrical threshold was the lowest voltage which elicited a perceptible contraction of the gastrocnemius. Two stimulators were employed in different experiments. One was designed and built by Mr. Craig Goodwin and is somewhat similar to that reported by Dusser de Barenne, Garol and McCulloch (5). It delivers a stimulus having an inverted saw-tooth wave form, and voltage, falling phase and frequency can be controlled stepwise. The other was a transformer delivering 60 cycle a.c. of controllable voltage. Thresholds of the several points on the circulated nerve were recorded at 5 or 10 minute intervals for a period of from 15 to 130 minutes. Then the trachea of the animal was clamped and observation of thresholds at the same points was continued usually until the

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motor response was no longer elicitable from any of the stimulation points. The nerve was kept moist with warm (38°C.) 0.9 per cent NaCl solution, and the preparation as a whole was maintained at 29°–32°C.

When amplified action potentials were observed oscillographically the preparation was similar. Only one root was used. The branches to the medial and lateral heads of the gastrocnemius were severed close to the muscle, and the medial popliteal nerve was cut at the same level. The nerves were crushed near their cut ends. Stimulating electrodes were moved along the nerve. The stimuli were derived from a stimulator synchronized with the sweep which was operating at a frequency of 60 per second. Action potentials were recorded monophasically either from the combined medial popliteal and branches to the gastrocnemius or from the latter alone. Functional status of the larger fibers was estimated from values for threshold stimulus intensity for A-wave elicitation, height of maximal A-spike, and stimulus intensity required for one-half maximal A-spike. After deterioration had progressed the positions of both recording and stimulating electrodes were altered to test segments of the nerve. The entire cat was kept inside a transparent plastic case. The atmosphere within was maintained at 35°–38°C. and saturated with water. The nerve was moistened occasionally with warm Ringer's or NaCl solution.

## RESULTS

With the muscle as an indicator of motor nerve activity, initial electrical

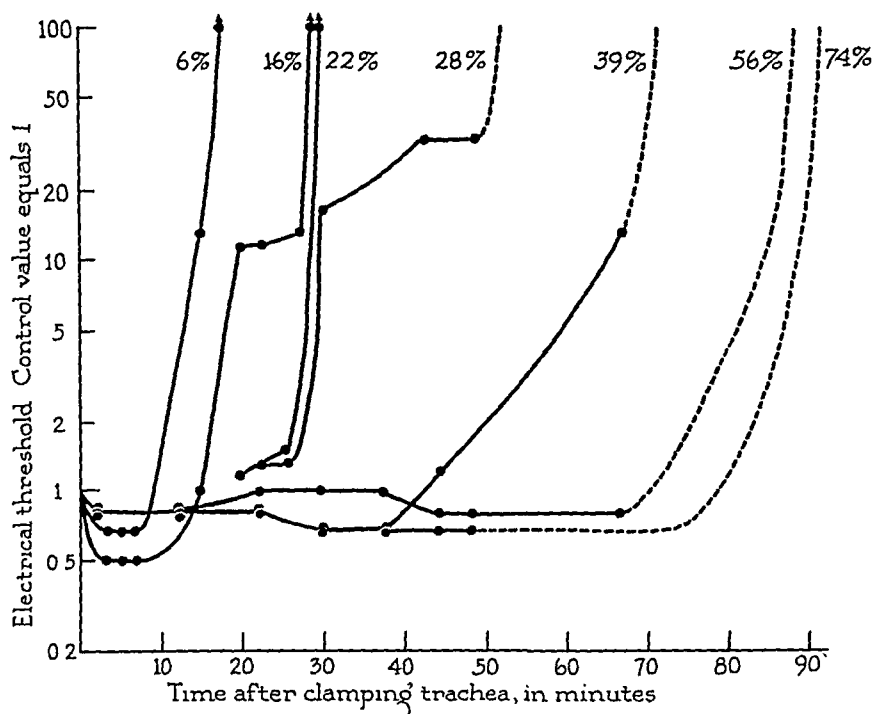


FIG. 1. Chart showing threshold changes at several levels along the nerve in a single experiment in which muscle contraction served as the response. The label on each set of points gives the position of the stimulating electrode along the nerve in terms of its distance from the spinal cord expressed as per cent of the total length of the nerve. The 6 per cent and 16 per cent levels are on the root. The terminal broken-line portion of some curves is based upon data from other experiments. Points are connected principally for clarity of presentation. Ordinates are on a logarithmic, abscissae on a uniform scale.

thresholds at different points along the nerve were practically the same in a given preparation. No significant alteration of thresholds occurred during the control period which in some experiments was as long as 130 minutes. The threshold values ranged from 0.01 to 0.06 volt among the animals. The essential results of clamping the trachea can be seen in Figures 1 and 2. The

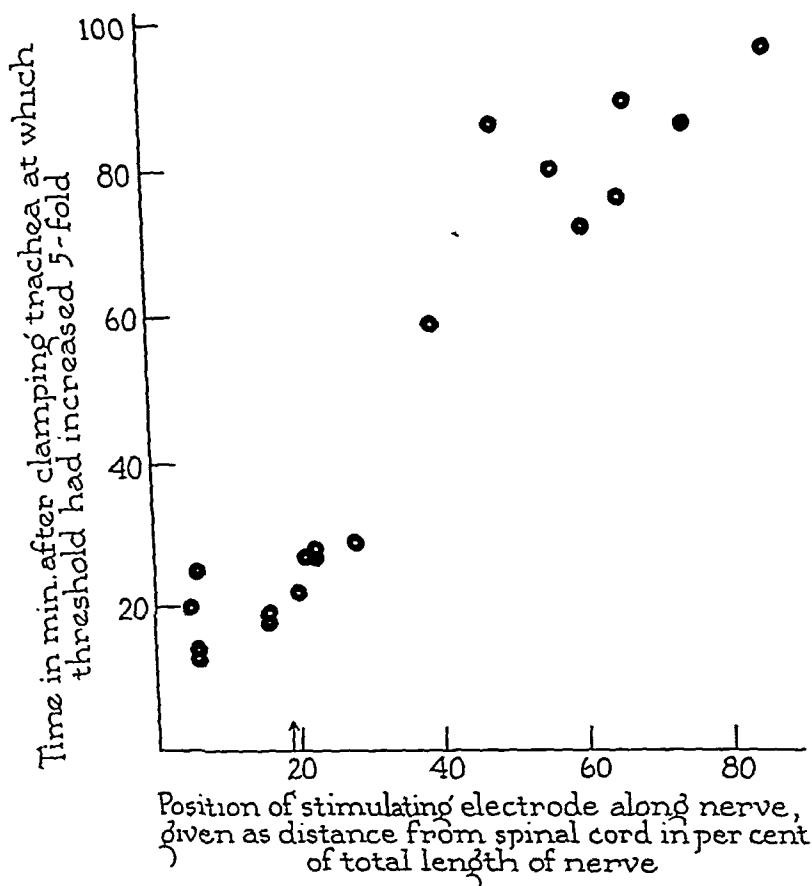


FIG. 2. Composite data from several experiments in which muscle contraction served as the response. The slope of the line joining points of each experiment is always positive; *i.e.*, within each experiment the proximo-distal gradient is unbroken. The arrow indicates junction of ventral root and peripheral trunk.

most proximal point on the nerve was invariably the first to undergo a rise in threshold and the first to become completely inexcitable. With passage of time, successively more distal points underwent elevation of threshold progressing to complete inexcitability. The character of the gradient of deterioration in the proximal 25-40 per cent of the nerve differed from that in the distal 60-75 per cent. At a given point in the proximal segment, the



time interval between the first rise in threshold and complete inexcitability was short. Two or 3 cm. distal to a point of nearly complete inexcitability there was little or no change in threshold. In the distal segment, however, the rate of threshold elevation usually was low for a time and points of moderately elevated threshold occurred along 5-6 cm. of nerve. Then the threshold rise usually became more precipitous and widely separated points became completely inexcitable within a short time span.

The general character of the proximo-distal gradient of nerve deterioration was confirmed with the cathode ray oscilloscope. It was also shown that at 120 minutes after clamping the trachea, with deterioration well advanced, there was less deterioration in the 35 mm. segment, the extremities of which were approximately 60 per cent and 80 per cent of the distance from proximal to distal end of the nerve, than in the adjacent proximal 35 mm. stretch. In addition it was found that the slower conducting motor fibers in the ventral roots deteriorated before the faster ones, and in the trunk the faster fibers survived longest. (The C wave was not under observation.)

The maximal response to stimulation at a give point could be observed to decrease gradually after the threshold began to rise, though it was measured only in the oscillographic experiments.

#### DISCUSSION

Gradients along the nerve or differences between ventral roots and peripheral trunks have been reported before. In Heinbecker's (12) experience ventral roots of the frog were in general depressed more quickly by oxygen lack than were the nerve trunks. Nachmansohn (18) reported figures indicating that in the dog choline esterase is more concentrated in ventral roots than in sciatic nerve. Grenell and Burr (11) found that under various conditions a bio-electric potential gradient exists along a peripheral nerve; distal points are negative to proximal ones.

Parker and Paine (19) found evidence of a proximo-distal progression of histological degeneration in the cut lateral-line nerve of the dogfish. The studies of Rosenblueth and Dempsey (22) proved that functional failure in Wallerian degeneration follows a centrifugal course in the medial and lateral popliteal nerves of the cat. Rosenblueth and del Pozo (21) confirmed and extended this work, finding that distal cuts additional to the primary one at the hip did not alter the course of degeneration which thus appeared to occur along a single, intrinsic gradient for the nerve as a whole.

Lewis, Pickering and Rothschild (16) arrested the bloodflow to the upper limb of humans by means of a pneumatic pressure cuff, and also deprived nerves of their circulation locally by means of a special pneumatic clamp which allowed blood to circulate in the remainder of the arm. The sensory and motor paralysis which developed began first in the fingers and spread up the arm. This centripetal paralysis was related, in the experiments with the special clamp, to the length of a nerve fiber that existed between its peripheral termination and the ischemic segment. The conclusion was that

nerves become more sensitive to ischemia as they are traced back from their endings toward the central nervous system. This would appear to constitute evidence gathered from the more peripheral part of the trunks, of the same gradient which we have demonstrated. In this connection it may be pointed out that the gradient in the trunk as demonstrated by us is valid not only for motor fibers but for the A-fibers collectively.

It is very likely that all or most gradients along a nerve will be explainable on the basis of the centrifugal flow of endoneurial fluid which Weiss and co-workers (26) have demonstrated and the perpetual peripheral movement of axoplasm from the cell body for which Weiss has found strong circumstantial evidence (24, 25, 26).

It seems most likely that cessation of the circulation of the nerve preparations studied resulted in their loss of excitability due to blocking of nerve fibers preceded by depression. Results indicate that in this situation a given block along a given fiber must be thought of as involving but a very short segment of the fiber. The fiber becomes blocked along a greater length when many of these short blocked segments fuse. Whether or not this punctate blocking occurs in strictly random fashion within a given fiber diameter group in the cross section of the nerve is immaterial at present. Our results show, however, that the blocks are not randomly distributed along the peripheral length of the motor nerve fibers collectively, but rather occur first at the end of the roots next to the spinal cord. With the passage of time, blocks become distributed farther and farther peripherally. The length of nerve in which blocking is occurring at a given time may be called the field of blocking. This field of blocking, then, originates at the central end of the nerve and moves peripherally. Within the field of blocking, blocks tend to appear most rapidly at the proximal end, least rapidly at the distal end, with a gradient between. Thus it is that the level of complete functional nerve block progresses peripherally, and below the point above which all the fibers are blocked in their entire length, the cross-sectional number of blocks per unit number of fibers at any given time diminishes progressively in the peripheral direction.

In the proximal 25-40 per cent of the nerve, over which the field of blocking spreads in the earlier part of deterioration, the field is relatively short in length. This follows from the fact that a relatively steep gradient of excitability exists in this locality so that a level at which the nerve is completely inexcitable is separated by but 2 or 3 cm. from a part which is normally excitable. In the distal 60-75 per cent of the nerve the field of blocking becomes longer and the gradient within it less. This is indicated by the fact that in the later phase of deterioration when blocking is occurring in the distal portion of the nerve, the front of the advancing wave of depression of excitability covers a much greater length of nerve, and when excitability at a level 50 per cent of the total nerve length from the origin is severely depressed, it may be moderately depressed at a level much nearer its distal end. The lengthening of the field of blocking toward the periphery is a natural

consequence of some variation in gradient or in susceptibility among the larger, longer surviving fibers constituting the principal group under observation. The deterioration gradient of which we write, then, is very noticeable in the proximal 25-40 per cent of the nerve, but less easily demonstrable in the distal 60-75 per cent.

There are two other hypothetical patterns of blocking in a non-circulated nerve which would result in a proximo-distal gradient of threshold elevation in our experiments, but they are incompatible with the facts. There would be a threshold gradient in the absence of a deterioration gradient if punctate blocks occurring with passage of time were randomly distributed along the length of the nerve and either randomly within a given fiber-diameter group

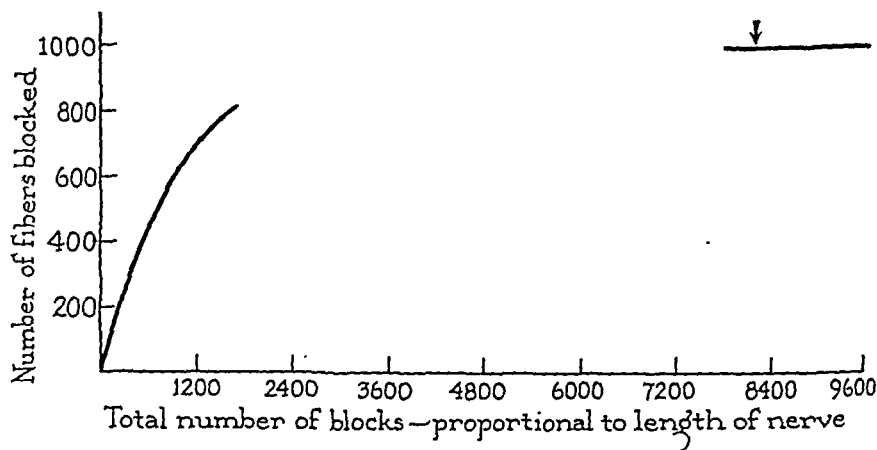


FIG. 3. Illustration of a hypothetical situation in which punctate blocking occurs with time randomly along the length and width of a segment of nerve composed of 1000 fibers. The abscissa of the point marked by the arrow is the total number of blocks which has occurred at the earliest time at which all fibers are blocked at some point in their course.

along the width (*i.e.*, in the cross-sectional area) of the nerve or in a pattern conditioned only by the position of the fibers in the cross-sectional area. The proximo-distal threshold gradient would exist because at a given time progressively fewer blocked fibers would intervene between stimulus and muscle as the stimulus was moved along the nerve toward the muscle. What this gradient would mean in terms of changing thresholds along the deteriorating nerve can be derived from Figure 3. Points on the curve were calculated on the basis of the probability of each punctate block as it occurs blocking a previously unblocked fiber. At a given time the total number of blocks in any segment of the nerve will be proportional to the length of that segment. It can be seen that when, in an arbitrary segment of a nerve containing 1000 motor fibers, a time is first reached such that all the fibers between the extremities A and Z are blocked at some point in their course, then 98 per cent (or only slightly higher if 10,000 or 100,000 fibers are as-

sumed) of the fibers will be blocked between the mid-point M of the segment and either extremity. Or, when only 50 per cent of the fibers are blocked between A and Z, 30 per cent will be blocked between M and an extremity. The results of experiment do not satisfy these conditions. The threshold of the midpoint of the nerve tested is not elevated at a time when the roots are completely inexcitable. Moreover, deterioration in a partly deteriorated nerve is further advanced in the central than in the peripheral half of a segment.

There would be a gradient of deterioration as well as a threshold gradient along the nerve if each fiber began to block at its central end and became progressively blocked by the distal extension of this proximally blocked section so that, at a given time, each fiber would be solidly blocked proximal to a point, unblocked distal to the point. This possibility is negated by experimental finding. When stimulating electrodes are placed at a point A approximately 40 per cent of the distance from proximal to distal end of the nerve in which deterioration is well advanced, the A-wave threshold decreases and maximal A-spike potential increases when the recording electrodes are brought from a point Z on the nerve 70 mm. distal to a point M 35 mm. distal to the stimulating electrodes. This means that of the fibers blocked between A and Z some are blocked only between M and Z, which contradicts the hypothesis.

The cause of functional deterioration of the nerve in these experiments has not been investigated. However, since there was no gradient in experimental conditions along the nerve the fact remains that regardless of immediate cause of the deterioration its centrifugal course is a manifestation of a gradient that exists along the living nerve. It is doubtful that this gradient is morphological. Dunn (4) concluded that in the frog hind limb the nerve fibers taper as they pass peripherally. However, her belief was based on the diminution in area of the largest fibers passing distally along unbranched nerve. She also demonstrated increase in the number of fibers at the same levels due to splitting. In the light of the findings of Eccles and Sherrington (6) that splitting occurs most commonly among the largest fibers and that the daughter fibers are reduced in size, it seems that a proof of tapering in trunks is lacking. Dale (3) found that a slight reduction of fiber size in the absence of branching occurs in the centrifugal course of the elongated caudal ventral roots of the cat. The excellent work of Rexed (20) seems to furnish conclusive answer to the question of nerve fiber tapering. He found no tapering of fibers in 15 cm. lengths of human S1 ventral roots, nor in 10 cm. intrathoracic lengths of rabbit phrenic nerve extending distally to within 2 cm. of the diaphragm. He concluded that any diminution in diameter of nerve fibers toward the periphery is due only to branching in the vicinity of the muscle.

Eccles and Sherrington (6) observed that the motor fibers in L7 and S1 ventral roots of the cat fall into two size groups with peaks at  $15\mu$  diameter and  $5\mu$  diameter. The motor fibers in the nerve to the medial head of the

gastrocnemius fall into two groups with peaks at  $15\mu$  and  $6\mu$ . In this nerve the larger fibers begin to dichotomize at approximately 60 mm. from the muscle and the splitting increases as the nerve approaches its destination. The sum of the cross-sectional areas of the daughter fibers averages only very slightly more than the cross-sectional area of the parent fiber. It is to be recalled that in the present experiments the faster fibers at a given level in the roots or trunk survived much longer than the slower ones after death of the animal. In view of the decrease in fiber size in motor nerves in the proximity of their muscle, the further extensive decrease in fiber size when the nerve fibers reach the muscle, and the earlier deterioration of smaller fibers at a given level, it can be said that the centrifugal course of deterioration exists despite any reduction in fiber size toward the periphery.

Oxygen lack is the likeliest primary cause of the functional failure of the nerves in our experiments. The only oxygen available was that in the air to which the nerves were exposed. Gerard (7) states that the oxygen concentration in air is insufficient to supply the requirements of thick mammalian nerves near body temperature, and that even pure oxygen is unsatisfactory for very thick nerves or nerves not freely exposed. The early lowering of thresholds which we usually encountered is a phenomenon which previously has been observed to occur in nerves deprived of oxygen (10, 12, 13, 14, 23). Heinbecker (12) and Heinbecker and Bishop (13) found that the faster conducting fibers are affected later by asphyxia than the slower, another symptom of oxygen lack which our preparations exhibited. In addition, cat phrenic nerve deprived of blood but kept in oxygen at pH 7.4 and  $37^{\circ}\text{C}$ . remains in fairly good condition for 10–20 hours (15) and cat sciatic nerve separated from its perikarya shows functional deterioration only after 2 days have elapsed (22). Thus, it is improbable that substances in the nerve derived either from the blood or from the cell body (cf. 2) were exhausted in our experiments.

The gradient of deterioration that we observed may be related to the energy and oxidizing reserves upon which the nerve can exist for a time in the absence of oxygen (1, 7, 8, 9, 10). Either the energy requirements of the nerve decrease toward the periphery or the concentration of some or all of the reducible, hydrolyzable or otherwise expendable substances identified with the reserves increases toward the periphery. Favoring the second hypothesis over the first are the findings by Gerard (9) that the oxygen consumptions of the upper and lower halves of the frog sciatic are equal, and by Weiss and coworkers (24, 25, 26) that endoneurial fluid and perhaps also the axoplasm move toward the periphery. Such flow could result in a gradient of concentration of chemical substances. Other alterations which might contribute to the failure of the nerve but which are of doubtful importance in view of known facts and the experimental conditions include lowered pH, exhaustion of substrates, inhibition of energy metabolism by metabolites, and dislocation of enzymes, coenzymes, substrates or ions due to incipient deterioration from any other cause.

As has been mentioned previously, Wallerian degeneration follows a

centrifugal course (21, 22). According to data presented by Rosenblueth and del Pozo (21) the length of nerve, 4 cm., separating the nonfunctional central region from the functional peripheral part is approximately equal to the field of blocking of our experiments for the same level. This suggests a close relationship between the gradients in the two cases.

A further point of similarity between events in Wallerian degeneration and in deterioration of non-circulated nerve is the relationship between depression of function in the nerve and of neuromuscular transmission. According to Lissák, Dempsey and Rosenblueth (17), all stages of transmission other than the first were deficient before conduction was affected (cf. 22). In 4-day degenerated nerves transmission was usually totally absent while sizable A-spike potentials could be recorded. In our experiments there was a suggestion that early failure of transmission often interfered with observations on the distal 50 or 60 per cent of the deteriorating nerve. In the extreme instances electrical thresholds on this length of nerve rose abruptly with little or no semblance of a gradient at a time when experience indicated that this segment of nerve should still function. The muscle was still excitable. The defect might have been due either to failure of neuromuscular transmission or to deterioration of the fine terminal motor branches, but the point was not studied.

#### SUMMARY

Electrical thresholds of points on a circulated nerve preparation in the cat were determined as the least electrical stimulus required to evoke either a contraction of muscle or the A-wave led from the distal end of the nerve. Then the trachea was clamped and threshold observations were continued.

The most proximal point was always the first to undergo an increase in threshold culminating in complete inexcitability. Successively more distal points underwent similar change with passage of time. Points 6 per cent and 85 per cent of the distance between proximal and distal ends of the nerve underwent a 5-fold threshold increase in 13 and 96 minutes respectively.

Roots were inexcitable before onset of threshold elevation at the midpoint along the nerve length. In a partly deteriorated nerve, deterioration was further advanced in the central than in the peripheral half of a segment, but of the fibers blocked in the segment, some were blocked only in the peripheral half.

It is thought most likely that in these experiments a given block along a given fiber involved but a very short segment of the fiber, blocking along a greater length resulting from fusion of these short blocked segments. The field of blocking originated at the central end of the nerve and moved peripheralward. Within the field of blocking, blocks appeared most rapidly at the central end, least rapidly at the peripheral end.

This proximo-distal gradient of deterioration must be a manifestation of a gradient along the intact nerve in the living animal.

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# HYPOTHALAMIC REGULATION OF SLEEP IN RATS. AN EXPERIMENTAL STUDY

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## INTRODUCTION

THE ESSENTIAL role of the nervous system in the regulation of the sleep-waking rhythm of higher animals is now widely recognized. No less a person than von Economo, however, drew attention to the fact that the phenomenon of sleep cannot be accounted for by a mere functional change of the central nervous system from its condition during the waking state. It is indeed an important fact that the function of sleep, instead of being characteristic of higher animals, is also observed in organisms which do not possess a central nervous system, and even in several vegetable species. It is therefore impossible to attribute this mysterious function to any special organ. Since all experimental work on sleep has hitherto been confined to mammals, chiefly to cats and monkeys, practically no data concerning the comparative physiology of this phenomenon are available. Nevertheless, it seems probable that during the phylogenetic development the function of sleep, together with many other mechanisms, was progressively centralized into the nervous system from which organ all changes, characteristic of sleep, were ultimately effected. This centralization proceeded so far that the alternation of wake and sleep seems to be governed in mammals by a circumscribed area of the central nervous system, capable of determining physical and psychical activities. The existence of this "centre" for the regulation of sleep is generally accepted by students of this subject. It has, however, given rise to a number of problems, concerned in the first place with the make-up of the centre, and secondly with its mode of action. Is there one single centre for the regulation of the sleep-and-waking rhythm, or must it be thought of as composed of two antagonistic parts, *viz.*, a sleep and a waking centre? And next, on what structures and along what paths does it primarily exert its influence? These questions have been so divergently answered by various investigators that a brief review of the current opinions seems essential.

The first to recognize a central representation of sleep was the Viennese ophthalmologist Mauthner (32), who from his observations of many cases of Wernicke's disease and of "nona" (which was probably identical with von Economo's lethargic encephalitis) concluded that the area surrounding the oculomotor nucleus was of special importance for the regulation of sleep. In later years evidence in favour of a central regulation of sleep was put forward by many clinicians, of whom von Economo in particular distinguished himself by his classical study of the Vienna epidemic of encephalitic lethargica (11). The localisation given by Mauthner has been little changed.



Thus von Economo considered a rather extensive area in the posterior and lateral walls of the third ventricle as the site of the regulating mechanism. A number of observations of cases in which this area was destroyed by tumour seemed to confirm the importance for sleep and waking, ascribed by von Economo to the walls of the third ventricle.

This view could be gained only by careful comparison of many clinical cases in which often very extensive lesions of the central nervous system existed. It is, therefore, not surprising that many investigators were, by less critical observation, led to other conclusions. Trömner (46) concluded from a case of narcolepsy, in which autopsy revealed an extensive abscess of the left thalamus, that the regulation of sleep was a function of the thalamus. In later years Spiegel and Inaba (43) adopted the same view on the basis of their experimental work on rabbits and dogs. The experiments of Ranson on monkeys (36), however, clearly indicate that the thalamus is of no special importance for the alternation of wake and sleep, as even extensive destruction of both thalami did not result in any abnormality of this function. On the other hand, bilateral lesions in the area of the mammillary bodies caused the same marked somnolence which is such an outstanding feature of epidemic encephalitis. These results are in accordance with the clinical view that the vicinity of the third ventricle plays a specific role in the regulation of sleep.

How does the central area (whatever its localisation) so strongly affect the state of our physical and psychical activities? This question has been very differently answered. According to Mauthner (32), the first to accept a central regulation of sleep, an inflammatory edema around the oculomotor nucleus would exert a pressure on the important sensory pathways passing through the midbrain, thereby interrupting the corticopetal flow of impulses and thus causing sleep. From this conception it is evident that the special importance ascribed by Mauthner to the environment of the oculomotor nucleus, was attributed by him only to the topographical relations of this area to the main sensory systems. Moreover, sleep, according to Mauthner, would result from an isolation of the cortex from the outer world, and this opinion has received support from various authorities including Spiegel and Inaba (43), who based their concept on cases of somnolence which they obtained by inflicting lesions to the thalamus. The same stand is taken by Kleitman and Camille (30), who, for instance, claim that imperfect relaxation of the skeletal musculature may cause insomnia by keeping up a continuous stream of proprioceptive impulses to the cortex. An essentially similar opinion was expressed by Trömner in 1912. Whereas Mauthner and others apparently considered various unspecific factors (edema, exhaustion, etc.) the cause of the sensory interruption, Trömner accepted the concept of a nervous centre capable of blocking the sensory relaying centres of the thalamus.

The idea of a nervous centre exerting an active influence on the sleep-and-waking rhythm, thereby formulated for the first time, has since received considerable support, although the details of Tromner's concept of this centre, *viz.*, its localisation in the thalamus and its action via sensibility, have been effectively criticized by all the most competent investigators.

In 1918 von Economo published his report on encephalitis lethargica. In those cases of this disease in which somnolence and ophthalmoplegy were the main symptoms, inflammatory lesions were regularly found in the posterior wall of the third ventricle, extending backward to the level of the oculomotor nucleus. In other cases insomnia was observed, together with chorea. These symptoms von Economo ascribed to inflammation of a more rostrally situated part of the hypothalamus, the tuberal region, and of the adjacent portion of the striate body. The objection that these disorders of sleep might be the result of some toxic influence of the inflammation was rejected by von Economo as the encephalitic sleep was promptly reversible and its interruption did not leave any signs of defective mental lucidity, as would have been the case with intoxication. Therefore von Economo is convinced that the affected areas constitute a specific centre as postulated by Trömner. From the contrasts between the somnolent and the sleepless form of epidemic encephalitis, he concluded that the "Schlafsteuerungszentrum" consists of at least two parts (Fig. 1). The conception lay near at hand that the caudal part (inflammation of which caused somnolence) is essentially a waking centre, while the rostral part must for an analogous reason be supposed to act as a sleep centre.

Sleep, according to von Economo, would result from inhibition of thalamus and cor-

tex by the "Schlafsteuerungszentrum." However, von Economo does not agree with previous workers that sleep is brought about by neutralization of sensory stimuli, because his patients suffering from encephalitis lethargica did not show any decrease of sensibility. The most important element of von Economo's opinion, shared with Trömmner, is his belief that sleep is caused by active nervous inhibition of different parts of the central nervous system. This view, however, is contradicted by Ranson and his collaborators.

In the course of Ranson, Barris and Ingram's experiments on cats (27, 37) and Ranson's on monkeys (36), in which they inflicted lesions of great diversity to the hypothalamus, they observed many cases of somnolence following lesions of a well-defined hypothalamic area (which has been referred to on p. 304), but never were able to produce sleeplessness. In their 1939 review of the hypothalamus Ranson and Magoun make the following statement, which illustrates their point of view: "There is no good reason to believe that there is a subcortical centre, which, when active, inhibits the cerebral cortex and causes sleep; but there is abundant evidence that some structure or structures in the region of the third ventricle or aqueduct play an important part in maintaining the waking state, because lesions in this region cause somnolence." By theoretical deductions Salmon (42) arrived at the same opinion.

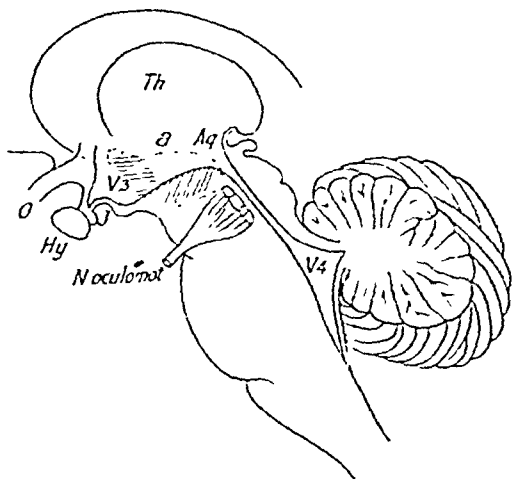


FIG. 1. Von Economo's conception (11) of the localisation of the "Schlafsteuerungszentrum" and its composition in two parts, viz., one (vertically striped) in which inflammatory lesions cause somnolence, and one (horizontally striped) in which similar lesions cause sleeplessness.

The experiments of Hess (23) have often been brought forward in favour of the existence of a sleep centre. By means of a special technique this observer was able to induce sleep in cats by stimulation of points in fore-, tween- and midbrain. These points lay widely scattered over so large an area that it is scarcely believable that Hess invariably stimulated one single centre. Nevertheless, the fact that sleep was obtained by stimulation, i.e., by activation of central nervous tissue, is deserving of our interest. Hess' method of stimulation, however, met with serious criticism from Harrison (21), who claims that it gives rise to electrolytic lesions of the stimulated points. When he altered it so as to avoid destruction of nervous tissue, he never was able to induce sleep. He therefore concludes that sleep in Hess' experiments produced not by stimulation but by lesion. This opinion, however, does not harmonize with the relatively short duration of sleep in Hess' experiments. Whereas the lethargy exhibited by the experimental animals of Ranson and his collaborators persisted for days and weeks, Hess' cats only

slept for a few hours. Probably this discrepancy should not be attributed to differences in the amount of nervous tissue destroyed, as even enormous lesions of fore- and tweenbrain fail to interfere with the regulation of sleep if only a circumscribed area of the hypothalamus is left intact. Nevertheless, Harrison's criticism warns us to be reserved in our appreciation of Hess' early findings. Another reason is that in later years Hess seems to have changed his initial opinion. In 1936, for instance, he stated that sleep could never be brought about by stimulation of the hypothalamus, but constantly appeared when the zone of transition between thalamus and subthalamus was stimulated. In 1944 he published some cases of "adynamia" in cats after hypothalamic stimulation, a condition which he distinguished from the sleep referred to in his previous papers by a concomitant plasticity of the animals. Hess' views on the diencephalic regulation of activity have been expressed in a recent review of his results (26), but his conception that sleep may result from activity from some central area still needs confirmation.

We shall not go into the experiments in which disorders of sleep were caused by the introduction of various chemical substances into the brain-stem, as their interpretation is too ambiguous to bring us any nearer to a satisfactory concept of sleep.

Looking back on the various opinions about the central regulation of sleep, a considerable amount of agreement now seems to have been reached as to the localisation of the centre of regulation. About its mode of action, however, some fundamental differences of opinion exist. Whereas von Economo agrees with Trömmner's original view that sleep results from activity of the regulating centre, which can therefore be thought of as a sleeping centre, Ranson and his school are by their experiments led to the view that the regulating apparatus essentially serves for the maintenance of the waking state, and that sleep appears when it is reduced to inactivity. In an experimental study described below, we hope to reach some conclusions which may contribute to the solution of the problems mentioned in the previous account.

#### MATERIAL AND METHODS

Only adult albino rats were used in this study. In these animals the effects of experimental lesions of the hypothalamus and adjacent parts of the brain on the regulation of sleep were observed. The choice of the rat as an experimental animal was forced upon us by war conditions and more especially by the impossibility of feeding larger animals during a sufficiently long period. Because of its small size, it was certainly not the most suitable animal for our purpose.

The various functions of the hypothalamus have hitherto chiefly been investigated either by the method of electrical stimulation or by causing extensive destruction of this part of the brain. Much of our knowledge concerning the lower parts of the brainstem has been obtained by a different method, *viz.*, the infliction of sharply incised wounds, and it is a striking fact that this procedure has scarcely ever been used in the study of hypothalamic functions. It has been followed only in the research of descending hypothalamic connections by Beattie, Brow and Long (3), and, more recently, by Magoun, Ranson and Hetherington (31). The method was considered a desirable addition to those procedures by which gross lesions of nervous tissues are brought about. It is probably the most suitable method for the tracing of pathways involved in the function under consideration, the damage to the brain being chiefly confined to interruption of fibre connections.

Unfortunately, however, it is still an open question whether the results of any destruction of brain tissue should be attributed to the exclusion of the destroyed or isolated area or to irritation of adjacent parts. Both conceptions can be and have been defended. The fact that many results of lesions of the central nervous system tend to subside in the course of time is often advanced in favour of irritation. It should be stressed, however, that the remarkable recuperative power which is exhibited by autonomic functions after lesion of the brainstem or spinal cord should in all probability be ascribed either to compensatory activity of those centres which subserve the same functions on a lower level ("automatisme étagé") or to the formation of new centres in adjacent regions. As far as the hypothalamus is concerned, there is another reason to doubt the irritative nature of incised wounds. Whereas unilateral electrical stimulation of this part of the brain is sufficient to cause widespread effects, which are eventually markedly bilateral (e.g., pupillary dilatation), incisions in the hypothalamus proved to be effective in our experiments only when bilateral. The sole stimulatory effect observed was a bilateral pupillary dilatation which occurred regularly during the unilateral introduction of the cutting instrument in the posterior part of the hypothalamus, and persisted for a few minutes after it had been withdrawn. Since the pupillary dilatation would, from various observations (38), seem to be a very constant reaction to hypothalamic stimulation, we could not escape the impression that the irritative action of an incision in the hypothalamus, if it exists, is of short duration. All postoperative effects were therefore interpreted as the results of exclusion of certain parts of the brain and not to irritation of the area surrounding the lesion.

For operative purposes the hypothalamus can be reached in several ways, the most usual of which are the subtemporal and the parapharyngeal approaches. Because of the bilaterality of the lesions which are required to obtain disturbances in the regulation of sleep, the subtemporal approach was unsuitable for our purpose. The parapharyngeal method, by which the hypothalamus is reached through the base of the skull, was greatly interfered with by the flat, expanded hypophysis and its encircling blood vessels. In the experiments described in the following paragraphs, transverse lesions of the hypothalamus were inflicted via perforation of the convexity of the brain. Naturally this primitive method, apart from the important advantage of a better general postoperative condition, has a number of disadvantages, the most important of which is the fact that it gives rise to considerable damage to structures which are situated dorsal to the hypothalamus. In fact, this damage is such that the incisions, instead of being restricted to the hypothalamus, extend throughout the dorsoventral diameter of the brain, so that many sagittal fibre connections in neocortex, archicortex and thalamus are interrupted as well as the longitudinal systems of fibres within the hypothalamus. The objection that many postoperative symptoms might be the result of this additional damage is therefore not unreasonable. Consequently, a number of controls was needed to decide whether or not the structures overlying the hypothalamus are involved in the regulation of sleep. These experiments will now be described briefly.

## RESULTS

In a number of animals an incision was made in one of various frontal planes between the anterior and posterior commissures, measuring from  $2\frac{1}{2}$  mm. on the right to  $2\frac{1}{2}$  mm. on the left side of the median plane, and not extending beyond the ventral border of the thalamus. These wounds corresponded with the most serious accidental lesions encountered (Fig. 2). Animals treated in this way did not develop any disturbances of the sleep-waking rhythm; they rapidly recovered from the operation. It is proved by this observation that *a normal alteration of wake and sleep is kept up so long as the transverse incisions leave the hypothalamus intact*. It does not, however, exclude the possibility that the relevant dorsal structures play some part in the regulation of sleep.

In another group of animals identical lesions were made, but on one side the incision was prolonged to the base of the brain. Consequently only the opposite hypothalamus was left intact (Fig. 3). Although the postoperative

mortality among these animals exceeded that of the first group, most of the rats made a rapid recovery. None of them displayed any disorder of sleep. Obviously one intact hypothalamus is sufficient for maintenance of normal sleep rhythm. The reverse experiment was carried out on a third group of rats. In these animals a combination of bilateral section of the hypothalamus and unilateral section of the dorsal structures was obtained by the introduc-

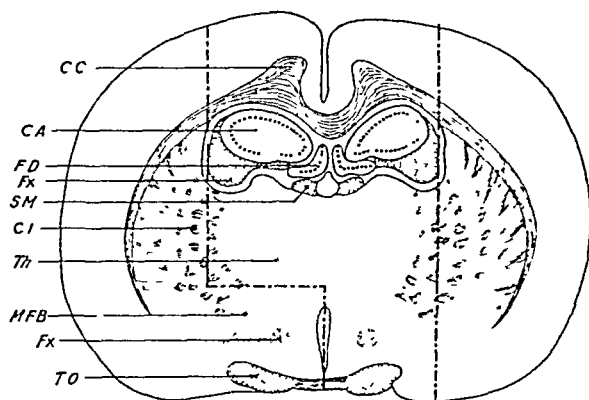
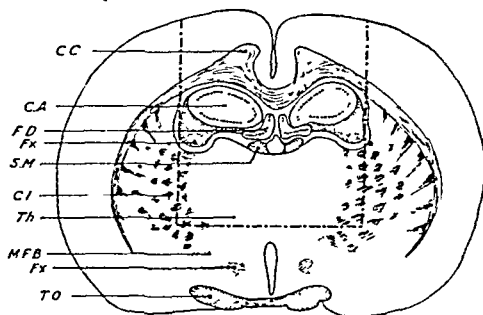


FIG. 2. Bilateral transection (indicated by interrupted lines) of the structures overlying the hypothalamus. C.A., cornu ammonis; C.C., corpus callosum; C.I., internal capsule; F.D., fascia dentata; Fx., fornix; M.F.B., medial forebrain bundle; Th., thalamus; T.O., optic tract.

tion with a "tour de maître" of a small hook-shaped knife, into the hypothalamus depicted in Figure 4. All animals treated in this way developed marked disturbances of the sleep-waking rhythm, which were identical

FIG. 3. Bilateral transection of dorsal structures and unilateral transection of hypothalamus. The lesion is indicated by interrupted lines. Abbreviations: see Fig. 2.



with those observed in cases in which bilateral lesions of the structures overlying the hypothalamus were inflicted.

From these observations it is evident that the dorsal structures (neocortex, archicortex and thalamus) of one side are unable to keep up the normal regulation of sleep. The disorders of sleep observed after transverse hypothalamic incisions from above may therefore safely be attributed to the lesions of the hypothalamus and not to the damage to more dorsally situated structures.

As to the operative technique, the operations were all carried out under ether anaesthesia and with aseptic precautions. After median incisions of the skin and periosteum,

the latter was pushed aside, and a uni- or bilateral trephine hole of about 4 mm. diameter was made just lateral to the median line, in order to avoid the superior longitudinal sinus. The dura mater was left in situ. In those cases in which the incision was intended to extend to one single lesion through both hypothalami, the small knife, depicted in Figure 4, was used so as to restrict the additional damage to one side. In cases in which it was essential to leave a medial hypothalamic zone intact—in other words, to restrict the lesions to both lateral hypothalamic areas—two separate incisions were required, and consequently the additional damage to the dorsal structures was inflicted on both sides. In the foregoing

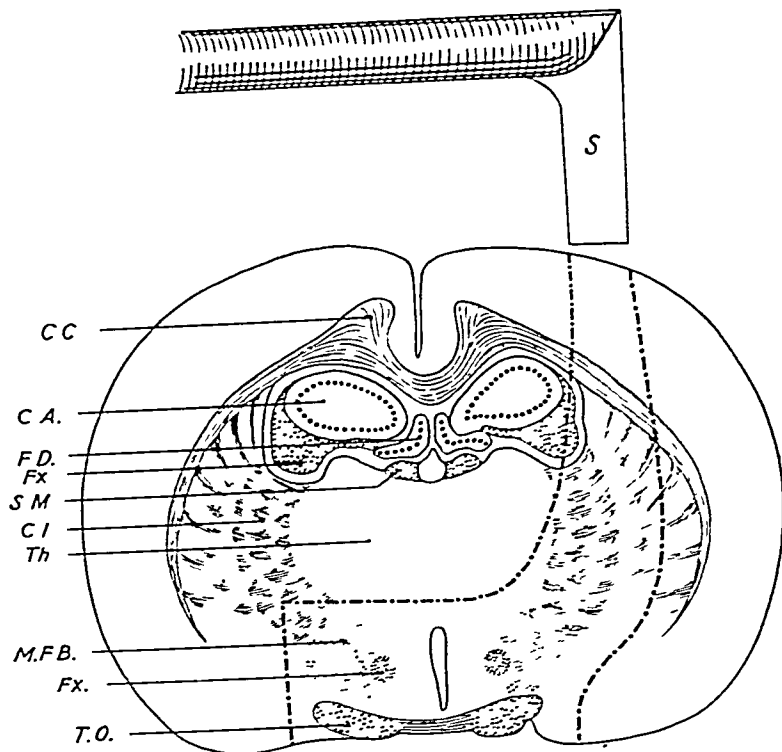


FIG. 4. Unilateral lesion to dorsal structures and bilateral transection of the hypothalamus (interrupted lines). The instrument with which the lesion was inflicted has been indicated with S. For abbreviations see Fig. 2.

account it has already been pointed out that these uni- or bilateral lesions to the structures overlying the hypothalamus had no apparent influence on the regulation of sleep.

After the lesions had been placed and the usual slight hemorrhage had stopped, the periosteum was stitched over the trephine holes and the skin was carefully closed. No dressing was applied. Throughout the operation 3 per cent hydrogen peroxide was used as an antiseptic. The anatomical investigation of the lesions was usually carried out after the animals had died from the results of the operation. When recovery took place the animal was sacrificed some time after the condition had become stationary. After their removal the brains were, as a rule, fixed in 96 per cent alcohol for three days and prepared according to one of Cajal's methods. Sagittal sections were found to be most suitable for the tracing of the transverse incisions. In the following account the disorders of sleep are grouped under two headings, *viz.*, disturbances of the waking capacity and those of the function of sleeping.

*Disorders of waking*

Certain bilateral lesions of the hypothalamus were found to cause a decrease in the waking capacity. The extent of this decrease varied in different cases.

(i) *Eight animals slept uninterruptedly so long as they were not roused by external stimuli.* They lay curled up on one side, breathing regularly, and could promptly be awakened by sufficiently strong stimuli, i.e., by pinching the tail or handling. When they were left alone afterwards, they would *yawn and stretch* and settle down in a comfortable position to go to sleep again. To strong stimuli the animals reacted vigorously, exhibiting all signs of intense emotion. There were no motor disturbances. Apart from the apparent inability to maintain the waking state, the animals displayed other autonomic disorders, which is not surprising in view of the many regulatory functions of the hypothalamus. All sleeping animals developed a marked hypothermia, which often was such that a rectal temperature of 25°C. was found after a stay of 24 hours in an environmental temperature of 18°C. The condition of sleep evidently did not depend on this hypothermia, as it was not less conspicuous in animals which were kept in an incubator of about 30°C. and consequently had rectal temperatures ranging between 35° and 40°C. Details of the temperature regulation cannot be given as a continuous registration of the temperature was not carried out.

The general condition of all hypothermic animals rapidly deteriorated; they constantly contracted a purulent conjunctivitis and rhinitis. To avoid these undesirable complications it was necessary to nurse the animals in a hot box. Moreover, extra attention had to be paid to their feeding. As many of the operated rats, especially the sleeping animals, did not take any food or drink of their own accord, it was essential to feed them artificially. For this purpose about 5 cc. of lukewarm, diluted skimmed milk was administered by tube (a soft catheter with a diameter of two to three millimetres) three times a day. In spite of all these precautions the animals never survived the operation for a long period. Only once was it possible to keep a rat (no. 55) alive during eleven postoperative days, the other animals dying after four to eight days. In rat 55 after the eighth day there were short periods during which the animal no longer lay curled up in its characteristic sleeping attitude, but sat huddled up with half-opened eyes, without, however, showing any spontaneous activity. It is an open question whether or not the capacity of waking is capable of a complete recovery in these sleeping rats. The animals could not be kept alive long enough and even the period of eleven days was too short to allow of an answer. From the results of the experiments of Ingram, Barris and Ranson (27) on cats and those of Ranson (36) on monkeys, it would seem that the capacity of maintaining the waking state does not completely return in these animals after optimal lesions of the hypothalamus. It is, however, improbable that the lesions in these animals were equivalent to those in ours. Future work on animals, better suited to this purpose than the rat, will have to solve the problem whether the total

loss of the hypothalamic regulation of sleep can be as satisfactorily compensated as, for instance, the thermoregulation after exclusion of the hypothalamus.

(ii) Another group of animals, instead of developing a complete condition of sleep, exhibited various degrees of drowsiness. They were inactive, and during the first days sat huddled up all the time, with eyes shut to slits. Like the other animals these rats could be roused promptly. After a period which lasted from one to three days, the somnolence tended to decline gradually, but in these animals also the general condition rapidly deteriorated and death resulted within a week so that the waking capacity was never observed to restore itself completely. Only occasional periods were observed in which the animals were in a somewhat more active condition, the eyes, for instance, being opened wider. It is of interest that these drowsy rats were never found to be in a typical sleeping state. Their condition was intermediate between waking and sleeping whenever they were observed. Like the sleeping rats they often developed a hypothermia and purulent infections of the mucous membranes and did not show any tendency to take food or drink of their own accord so that they too had to be nursed with tube feeding and hot box.

(iii) In a third group of rats the operation failed to produce any disorder of the waking capacity; neither did most of these animals develop any other serious disturbances of the general condition. In order to facilitate a survey of our material we abstained from verbal description of each case. Instead the lesions which were found in every single case were recorded into a diagrammatic horizontal section of the hypothalamus (Fig. 5).

The diagrams obtained in this way have been collected in three tables,

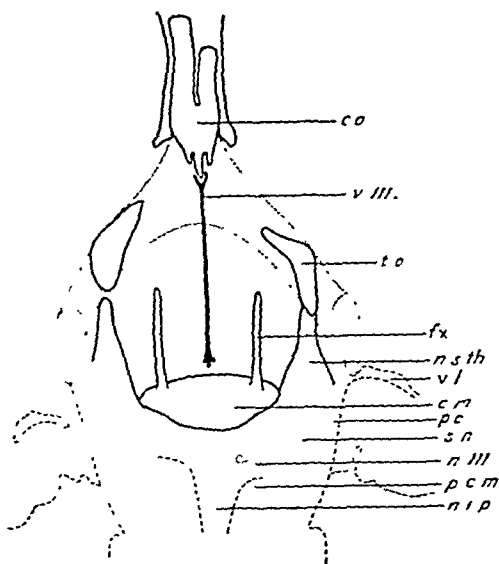


FIG. 5. Key figure to Tables 1-5. The diagram has been composed of two horizontal sections, of which one (solid lines) passes through the dorsal hump of the optic chiasma and through the mammillary bodies. The course of the optic tracts has been indicated by broken lines. The second section (outlines given in broken lines) lies on a more dorsal level and passes through the cerebral and mammillary peduncles (broken lines) and through the substantia nigra, the interpeduncular nucleus and the subthalamic nucleus, outlines of which are stippled. For Tables 1-5 only the basal section has been used. C.M., mammillary body; C.O., chiasma opticum; fx., fornix column; N.I.P., interpeduncular nucleus; N.S.Th., subthalamic nucleus (Luys); N.III, oculomotor nucleus; P.E., cerebral peduncle; P.C.M., mammillary peduncle; S.N., substantia nigra; T.O., optic tract; V.L., lateral ventricle; V.III, third ventricle.



*viz.*, Table 1, on which the findings in the completely sleeping rats are depicted; Table 2, showing the cases of drowsiness in as much of a descending order of intensity as was practically possible; and Table 3, which contains those cases in which the operation did not affect the waking capacity. The following points concerning the figures are of special interest.

(a) In all cases of characteristic sleep (Table 1) bilateral lesions were found which ex-

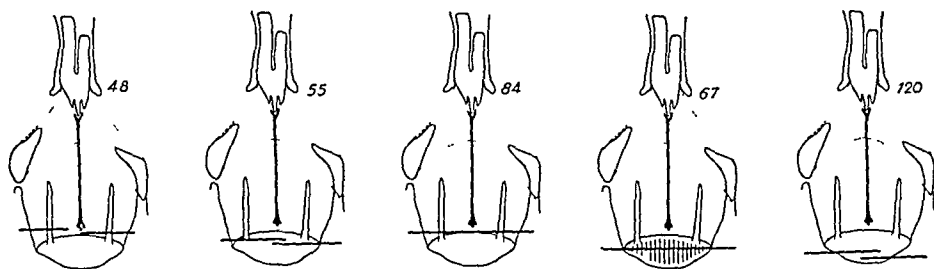


Table 1. Anatomical findings in five rats exhibiting a typical condition of sleep.  
For an explanation of the diagram, see Fig. 5.

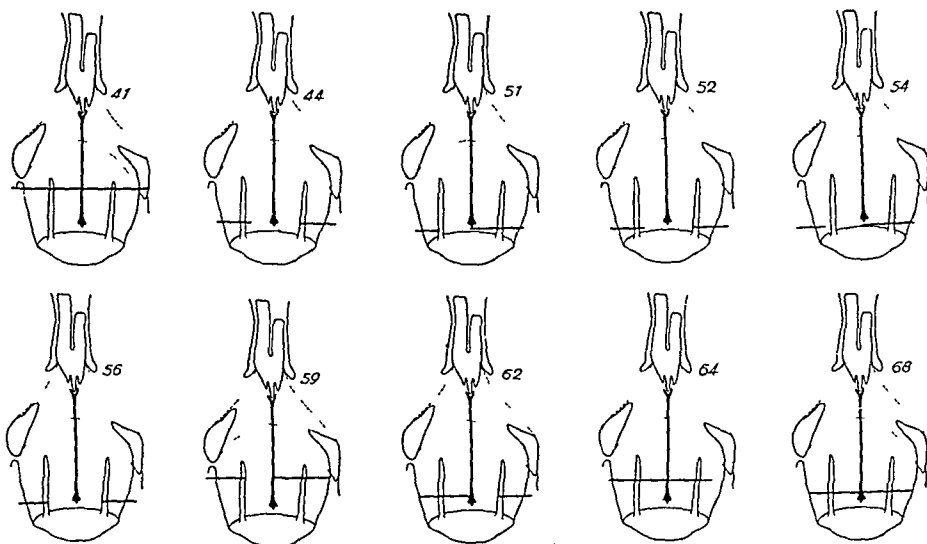


Table 2. Anatomical findings in ten cases of somnolence. See Fig. 5.

tended through the entire—or almost the entire (rat 48)—transverse diameter of both hypothalami. All these lesions were situated in the immediate vicinity of the mammillary bodies. In two of the animals the mammillary bodies themselves were involved in the lesions, in rat 67 these nuclear groups were largely destroyed by hemorrhage. In rats 48 and 84 the lesions were situated on the rostral border of the mammillary bodies, in three other cases, of which only rat 120 is shown, they were found slightly caudal to these cell-groups.

(b) Lesions of the mammillary region were also met with in some of the drowsy animals, collected in Table 2. These transections, however, differed from those found in the

first group in being *incomplete*. The region medial to the fornix column—comprising the periventricular and the medial hypothalamic areas according to Crosby and Woodburne (cited from 6)—had been left intact on both sides in rats 52 and 56, and on the left side in rat 51. In rat 54 the hypothalamic lesion on the left was confined to the lateral half of the lateral hypothalamic area, leaving the remainder of this area and both inner areas undamaged.

Yet this second group also contains a few cases in which a complete transection of both hypothalami was found. In these cases (rats 68 and 41), however, the lesion was situated farther rostrally than in the animals belonging to the first group. In rat 68, for instance, which was very drowsy, a complete transection of both hypothalami was found

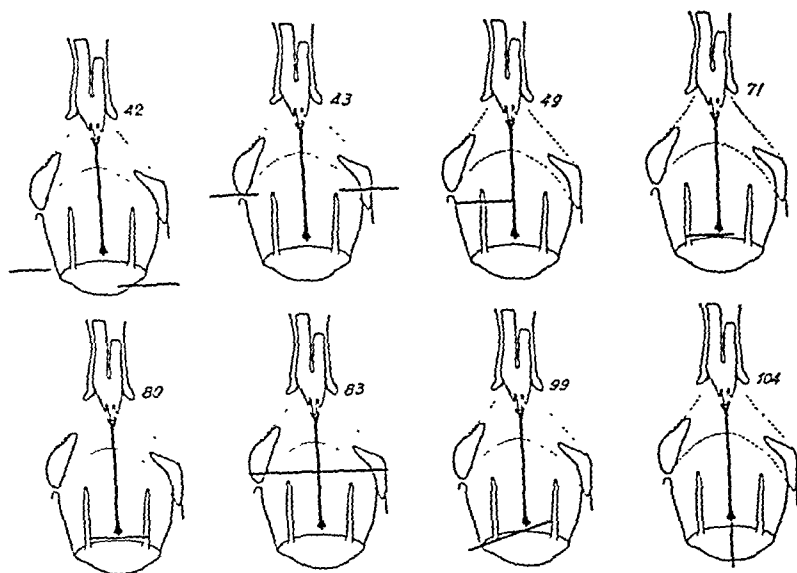


Table 3. Eight cases in which the operation failed to produce a decrease of the waking capacity. See Fig. 5.

about 1 mm. anterior to the mammillary bodies. Only a slight somnolence was displayed by rat 41; in this animal a complete bilateral lesion existed about 2 mm. in front of the mammillary bodies. As an identical lesion was found only 0.2 mm. farther ahead in rat 83 (Table 3) which showed no signs of somnolence, it would seem that the area in which hypothalamic lesions must be situated to cause a decrease in the capacity of waking has its rostral limit in a transverse plane about 2 mm. anterior to the mammillary bodies.

It has not been possible to ascertain the caudal limit of the area under consideration. The most posterior lesion which was found to disturb the waking capacity was situated slightly caudal to the mammillary bodies, i.e., in the tegmental region. More caudal lesions resulted in a loss of consciousness which was too little reversible to enable us to make any statements concerning the regulation of sleep in these animals.

(c) The third group (Table 3), containing a number of animals without any apparent disturbance of the waking capacity, is distinguished from the previous groups in various respects. In some of the animals (rats 42 and 49) unilateral lesions of the hypothalamus were found to exist. It has already been pointed out that unilateral hypothalamic lesions, irrespective of their location, are unable to affect the regulation of sleep. Apparently the same holds good for bilateral lesions which are confined to the medial part of the hypothalamus, viz., the zone medial to the fornix column. This is shown by rats 71 and 80, in which only the inner hypothalamic areas (the medial and the periventricular area) were damaged. It would seem to indicate that the medial part of the hypothalamus is of no special importance for the maintenance of the waking state. In the discussion of the second group it was mentioned, however, that the decrease of the waking capacity was less in

animals in which the lesions were limited to the lateral area of both hypothalami than in those in which, on the same level, the whole hypothalamus was bilaterally sectioned. This indicates that the inner hypothalamic areas do play a certain role in the maintenance of the waking state. We shall revert to this fact in one of the following sections.

The preponderance of the lateral area in the function of waking, already observed by Ranson (36) in monkeys, is clearly demonstrated by rat 99. No drowsiness was observed in this animal in spite of a transverse lesion in the mammillary region which was complete on the left and had been confined to the inner hypothalamic areas on the right. The lateral area of the right side was the only part of the hypothalamus intact, and it proved capable of maintaining the waking capacity. Evidently one lateral area of the hypothalamus is sufficient for maintaining this function. A lesion of exceptional location was encountered in rat 104. In this animal a median incision was found to exist, which reached the base of the brain between left and right mammillary body. Naturally it had severed the supra-mammillary commissure, which contains the crossing fibres of the hypothalamo-tegmental division of the medial forebrain bundle, in addition to crossing tegmental connections of the fornix column. The animal exhibited a slight drowsiness which entirely disappeared in the course of the first postoperative day. Incidentally, rat 83 was mentioned in the discussion of the second group. The complete bilateral transection of the hypothalamus, found in this case, was apparently situated too far rostrally to interfere with the waking capacity. It will again be reverted to in one of the next sections. A number of supplementary experiments have not been inserted in Table 3. In four rats extensive destructions of both thalami were brought about without causing the animals to show any decrease of the waking capacity. This does not harmonize with the findings of Spiegel and Inaba (43), mentioned in the introduction, and it offers confirmation of Ranson's observations (36).

In addition it should be stressed that bilateral transverse incisions on a level with the mammillary bodies only caused disorders of the function of waking if they involved the basal part of the brainstem, as, for instance, was the case in rat 120 (Table 1). If not extending ventrally beyond the central grey substance around the Sylvian aqueduct, these lesions, intermediate between diencephalon and mesencephalon, fail to interfere with the waking capacity. On this point our findings are in line with the results of Ingram, Barris and Ranson (27). From their experiments, in which somnolence was produced in cats by small bilateral lesions in the basal part of the tegmental area adjacent to the mammillary bodies, we may conclude that the function of waking, as far as it is performed by the midbrain, is localised in the basal part of this structure, *i.e.*, the tegmentum. The conclusions arrived at in the previous account indicate a specific importance of a certain area of the brainstem for the maintenance of the waking state. Certain lesions of this area cause a total loss of this function. In view of the arguments, advanced in the discussion of the operative method pursued in this study, we are inclined to consider this disorder to be a result of the exclusion of a certain centre, which therefore may be termed a waking centre.

#### *Connections of the region of the waking centre*

It was pointed out in the preceding section that the region formed by the posterior part of the hypothalamus and a hitherto undefined portion of the adjoining tegmentum mesencephali is likely to contain a waking centre. It is an important fact that the same region of the brainstem, according to the results of Beattie (2), Ranson, Kabat and Magoun (38) and others, is the site of the highest orthosympathetic centre. Its stimulation is followed

by a rise of blood pressure, an increase in rate and depth of respiration, pupillary dilatation, pilo-erection, etc., which give an appearance of intense emotional activity.

It has long been recognized that the autonomic balance lies relatively on the orthosympathetic side during the waking state and shifts to the parasympathetic side during sleep. If one combines this fact with the aforementioned results of stimulation, it does not seem impossible that there exists at least a partial identity between the waking centre and the orthosympathetic centre in the hypothalamus, and that the waking state is merely one of the manifestations of orthosympathetic activity. All orthosympathetic phenomena which result from stimulation of the hypothalamus can be brought about only by a descent of hypothalamic impulses to lower levels of the central orthosympathetic system, from where they are conducted along the peripheral orthosympathetic pathways to the various end-organs concerned.

Those somatic phenomena which indicate the shift to the orthosympathetic side during the waking state are certainly effected along this way. The most striking difference between wake and sleep, however, lies in the degree of consciousness. The question lies near at hand—if perhaps the increase of consciousness caused by the activity of the waking centre is brought about by a conduction of impulses via the same lower centres and the same peripheral pathways to the cerebral cortex. Moreover, there is still another possibility for the hypothalamus to stimulate the cerebral cortex, as stimulation of the hypothalamus brings about a production of adrenalin by the suprarenal gland, which tends to raise the level of consciousness.

Experimental evidence, however, seems to indicate that the peripheral autonomic system is not involved in the maintenance of the waking state. The observations of Cannon and his associates on cats in which practically the entire peripheral sympathetic system, including the medulla of both suprarenals, had been removed (8), have proved that under these circumstances a fairly normal condition can be kept up, so long as the exigencies of the outer world are held within certain limits. They apparently did not observe any change of the sleep-waking rhythm in sympathectomized animals. These facts render it highly probable that the waking centre does not affect the cerebral cortex along peripheral pathways but influences cerebral functions along central corticopetal connections.

At this point the question arises as to what ascending connections may account for the action of the waking centre on the cerebral cortex. As no data concerning this problem could be found in literature, we decided to study the fibre degeneration following lesion of the area in which the waking centre is probably located. For this purpose the mammillary area of the right side was sharply cut across in rat 66. The operation was carried out in the usual way, the wound extending from the dorsal surface to the base of the brain, and consequently the structures overlying the hypothalamus were also damaged.

As could be expected in view of the unilaterality of the lesion, the animal did not develop any disorder of the sleeping rhythm. It made a rapid recovery, and was killed ten days after the operation. Its brain was treated according to the prescription given for the Marchi technique by Romeis (40), and was embedded in paraffin via the graded alcohols and cedar oil, after which it was serially sectioned in the sagittal plane.

On microscopic investigation the location of the lesion was found to be as follows. The dorsal surface of the brainstem was reached at the habenula; after traversing this ganglion the instrument had apparently followed the rostral side of the habenulo-peduncular tract for some distance, but on a more ventral level the wound diverged from this bundle in a rostral direction so that it reached the base of the brain through the caudal one-third of the mammillary body (Fig. 6). In a transverse direction it extended from about 0.2 mm. to so far outside the median plane that it had severed the most medial fibres of the cerebral peduncle as its lateral end. Consequently, the whole transverse diameter of the hypothalamus had been cut, with the exception of a narrow medial zone of about 200 $\mu$  in width. As a result of this lesion a large part of the fibre connections between the hypothalamus and lower parts of the nervous system had been interrupted. Only those fibres running in the undamaged paramedian zone and including the medial part of the periventricular fibre system of Schütz had been left intact.

As could be anticipated in view of the damage to the mammillary body, degeneration was found in some of the fibre systems connected with this cell group. Vicq d'Azyr's

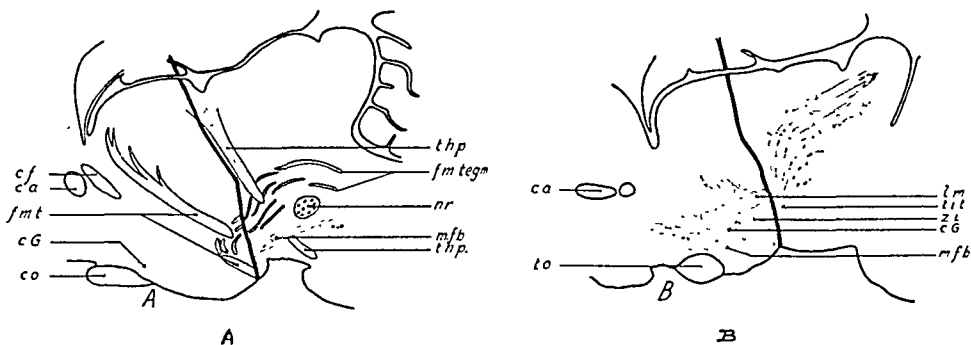


FIG. 6. Sagittal sections through the brain stem of rat 66, Marchi method, ten days after operation. Section A is situated 0.6 mm., section B 1.5 mm., lateral to the median plane. The lesion is indicated with a thick line. Descending (A) and ascending (B) degeneration of the medial forebrain bundle. c.a., anterior commissure; c.f., fornix column; c.G., Ganser's commissure; c.o., optic chiasma; f.m.t., bundle of Vicq d'Azyr; f.m.tegm., mammillo-tegmental fascicle; l.m., lemniscus medialis; m.f.b., medial forebrain bundle; n.r., red nucleus; t.h.p., habenulo-peduncular tract; t.i., incerto-tegmental fibres; z.i., zona incerta.

mammillo-tegmental tract was heavily degenerated. Some degeneration was also found in the mammillary peduncle, indicating the occurrence of mammillo-fugal fibres in this bundle, which conflicts with Papez' conception that the system is entirely mammillopetal in nature (34).

Dorsolateral to the mammillary body, in the space between this cell group and the substantia nigra, the lateral hypothalamic area continues into the midbrain tegmentum. Many fibres belonging to this hypothalamic area, chiefly constituents of the so-called medial forebrain bundle, run through this space and connect with tegmental centres. Presumably these fibres, originating in di- and telencephalon, form the first link of a much interrupted pathway to bulbar and spinal autonomic centres. Magoun, Ranson and Hetherington (31) have offered proof that these connections are not arranged into circumscribed bundles in the midbrain but are distributed over almost the entire cross section of its tegmentum. Previously Beattie, Brow and Long (3), working with the Marchi technique, had claimed that the chief descending connections of the hypothalamus course in or near the central grey substance surrounding the Sylvian aqueduct, a position which conforms to that of the periventricular system of Schütz. Probably Beattie *et al.* observed degeneration of this system which connects with the inner part of the hypothalamus, *viz.*, with the periventricular and medial areas. This is in line with the fact that the transverse cut which they inflicted to the hypothalamus was confined to these areas and did not in-

volve the lateral area. They consequently did not observe degeneration of the fibres descending from the lateral hypothalamic area, which are more numerous than those of Schütz's system and, from the results of Magoun, Ranson and Hetherington, would seem to be the chief transmitters of orthosympathetic impulses from the hypothalamus. In our experiment these fibres had been interrupted. By virtue of their degeneration a large number of them could be traced in a caudal direction. Figure 6A shows a rather condensed group of these degenerated fibres, running underneath the red nucleus and radiating in a dorsocaudal direction into the tegmentum. A considerable number, not shown in Figure 6, spreads over more ventral levels of the tegmentum. None of these fibres could be traced into parts lower than the midbrain. A fair number of degenerated fibres ran from the hypothalamus through the supramammillary decussation into the opposite side of the midbrain.

In accordance with the observation of Beattie, Brow and Long (3), a number of osmophilic granules was found in the dorsal longitudinal bundle, *i.e.*, the caudal continuation of the periventricular hypothalamic system of Schütz. As only the lateral part of this system had been damaged, it also contained many normal fibres. Apart from this descending degeneration, a number of degenerated periventricular fibres could be traced in a rostral direction. This proves the occurrence of hypothalamopetal fibres in the system of Schütz, which therefore should not be regarded as completely efferent with regard to the hypothalamus. Identical findings were reported in the opossum by Bodian (7).

A much more extensive ascending degeneration was found in the lateral hypothalamic area. From the lesion a great number of degenerated fibres could be traced rostralward, chiefly occupying the lateral part of the lateral area and obviously belonging to the medial forebrain bundle. On passing forward through the lateral hypothalamic area their number continuously decreased, which suggests a distribution of this ascending system in the hypothalamus. Only a few of the fibres were found to extend farther rostralward, into the septal region, and none could be traced to still higher levels. This ascending system in the medial forebrain bundle conceivably originates in the mammillary region, and farther caudally, in the midbrain tegmentum.<sup>1</sup>

By way of summary we are able to state that our Marchi experiment seems to prove the existence of ascending fibres in several bundles connected with the mammillary body and its adjoining structures, *viz.*, in the mammillo-thalamic bundle of Vicq d'Azyr and in the medial forebrain bundle. The degeneration found in the fornix column cannot give any information concerning the direction in which the fibres of this bundle lead, because both of its terminations—the hippocampal formation and the mammillary body—had been damaged.

In his careful study of the diencephalon of the opossum Bodian (7) did not observe any mammillopetal degeneration in Vicq d'Azyr's fascicle after lesion of this bundle. In contrast to Le Gros Clark and Boggon (9), he concluded that this system is entirely efferent with regard to the mammillary body, a conclusion which seems to be substantiated by Droogleever Fortuyn (15), who in a developmental study of the thalamus observed an outgrowth of fibres only from the mammillary body towards the thalamus and not in the opposite direction. The occurrence of hypothalamo- and septopetal fibres in the medial forebrain bundle has so far not been described.

<sup>1</sup> Although it has no apparent bearing on our problem, it is interesting to note that degeneration was found in Ganser's commissure. Fibres of this heavily myelinated system were found to pass rostralwards through Forel's field, whence they curved downwards to cross underneath the third ventricle to the left lentiform nucleus. This finding is in accordance with the recent observation of Glees (19) that Ganser's commissure contains fibres connecting the medial fillet with the opposite globus pallidus.

In conclusion it seems probable that the region in which lesions must be situated to cause a maximal loss of the waking capacity has efferent connections with the anterior nuclei of the thalamus, the lateral hypothalamic area and the septal region.

The next step to take is to decide which of these connections may transmit the impulses from the waking centre to the cortex. None of the aforementioned ascending connections can be traced to the cortex itself. If a corticopetal system originates in the waking centre, it must be supposed to relay in the thalamus, hypothalamus or septum—perhaps in two or all of these structures. It has been pointed out that destruction of the thalamus, despite the conceptions of Trömmner and of Spiegel and Inaba and others, is not

followed by any decrease of the waking capacity. Bilateral lesions of the septum also fail to bring about alterations of the sleep-waking rhythm, as is shown by rat 114 (Table 5). Moreover, in one rat, which did not show any tendency to somnolence, autopsy revealed a large abscess, which had destroyed the septal region and the anterior half of the thalamus on both sides. Naturally these observations do not exclude the possibility that the septum and the thalamus do play a certain role in the corticopetal transmission of stimuli from the waking centre. At most it proves that these structures are not the only relaying centres involved in this function.

It seems reasonable to accept an intensive relay of impulses from the waking

centre in the lateral hypothalamic area. The majority of the ascending fibres in the medial forebrain bundle ends here, and it is striking that the degree of somnolence is more or less proportional to the extent to which these ascending fibres have been interrupted, the most effective lesions being situated in the caudalmost part of the hypothalamus and involving its lateral area in which the fibres are contained. If the lateral area of one or both sides partly or completely escapes injury, somnolence is either slight or lacking (cases 64, Table 2; and 43, 80, 99, Table 3). Moreover, the number of ascending fibres severed naturally becomes smaller with more forward localisation of the lesions, and as was pointed out in one of the foregoing sections, the effect of the lesions decreases accordingly. (Cf. Table 1 with cases 68 and 41; Table 2.)

From the fact that a maximal loss of the waking capacity is as well brought about by lesions just in front of the mammillary body as by those closely behind it, it seems probable that the waking centre itself lies in the region caudal to the mammillary body, *viz.*, in the midbrain tegmentum.

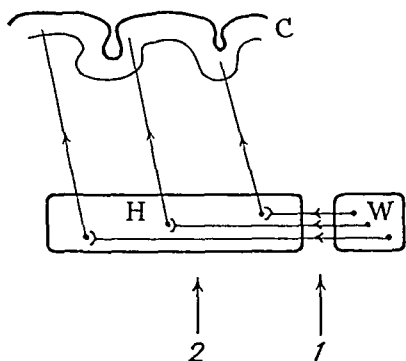


FIG. 7. Diagrammatic representation of the influence of the waking centre on the cortex, as suggested in the text. C., cerebral cortex; H., lateral hypothalamic area; W., waking centre.

We are inclined to suppose that it gives origin to a number of fibres which ascend through the narrow space between mammillary body and substantia nigra which should in this part of their course be considered to be the initial common path of the "waking" stimuli. Rostral to the mammillary body the termination of this system in the hypothalamus—chiefly in the lateral area of this structure—begins, and some fibres even extend farther forward into the septal region, as is demonstrated by our Marchi experiment. Our results seem to indicate that the fibres under consideration synapse in the hypothalamus, whence their stimuli are led off by secondary neurons sideways towards the cerebral cortex. It should, however, be stressed that hypothalamocortical connections, as suggested here, have never been morphologically demonstrated, and that consequently no statements concerning the course of this hypothetical system can at present be made.

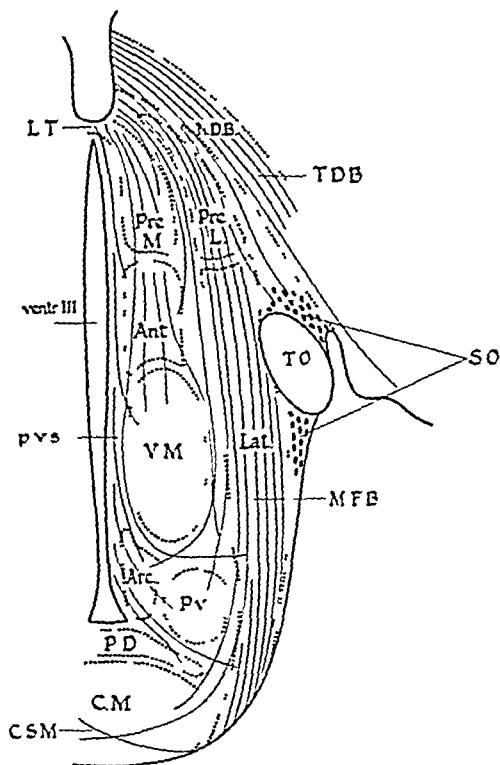


FIG. 8. A diagram of the longitudinal fibre systems in the hypothalamus, as seen in a horizontal section. Note the exchange of fibres between the lateral and the periventricular areas in the premammillary region. Ant, nucleus anterior; Arc, nucleus arcuatus; C M, corpus mammillare, C S M, commissura supramammillaris; Lat, nucleus lateralis; L T., lamina terminalis; M F B, medial forebrain bundle; N. D B, nucleus of diagonal band; P. D., nucleus praemammillaris dorsalis; Pre. M, nucleus preopticus medialis; Pre L, nucleus preopticus lateralis; P. v., nucleus praemammillaris ventralis; p. v. s., periventricular fibres; S O, nucleus supraopticus; T O, tractus opticus; T. D. B., diagonal band of Broca; ventr. III, third ventricle; V. M, nucleus ventromedialis.

Our concept is illustrated by Figure 7, which offers an explanation of the fact that a transection on a level with the mammillary bodies (arrow 1), interrupting the "initial common path," results in greater loss of waking capacity than a similar lesion in a more rostral plane (arrow 2). With more forward location of the lesion a larger number of fibres from the waking centre escapes injury. Finally, at about 2 mm. rostral to the mammillary bodies, their number already ended is sufficient to maintain a normal waking capacity, as is suggested by the absence of somnolence after transections on more rostral levels.

It has already been mentioned in the preceding section that the inner



part of the hypothalamus, although certainly of much less importance for the waking capacity than the lateral area, does seem to play a certain role in this function. Presumably a small number of fibres from the waking centre course in this part, which is formed by the periventricular and medial areas. It is true that no degeneration was found in these areas in rat 66, but it is possible that the fibres under consideration are unmyelinated. There certainly exists an extensive exchange of fibres between the lateral and the two inner areas, especially (Fig. 8) in the region just in front of the mammillary body, and it is possible that a number of ascending fibres deviate from the lateral area to continue their course in more medial parts of the hypothalamus. Possibly these fibres account for what remains of the waking capacity after lesions restricted to the lateral area.

### *Sleeplessness*

In comparison to the vast clinical and experimental literature concerning somnolence, astonishingly little is known about the opposite phenomenon: sleeplessness. It seems that only von Economo (11) has dealt with this subject in extenso. According to his observations inflammation of a rostral part of the hypothalamus, adjacent to the striate body, may result in insomnia. It would thus seem that the exclusion of the relevant hypothalamic area interferes with the function of sleep.

As far as we know, the only experimentally founded conception of insomnia is that of Ranson and Magoun (39) who in the course of many experiments on cats and monkeys never observed any influence of hypothalamic lesions on the capacity of sleeping. Their opinion about the regulating mechanism is sufficiently illustrated by the statement quoted in our introduction, from which it is evident that they deny the existence of a centre subserving the function of sleep in a restricted sense.

In the course of our own experiments we arrived at a different opinion.

(a) In a number of rats the lesion of the hypothalamus was followed by a condition of sleeplessness. After regaining consciousness some of these animals were restless and irritable, reacting vigorously to minor stimuli. Their condition closely resembled the sham rage observed by Fulton and Ingraham (16) in cats after prechiasmatic lesions, and described by Bard (1) as a result of decerebrations through the rostral part of the diencephalon. In a number of operated rats no such change of character was observed, the animals remaining as quiet as before the operation.

In both groups the normal alternation of wake and sleep had completely vanished. Naturally this fact could only be ascertained by means of a continuous observation of the animals. The normal difference in activity between day and night—established for the rat by Szymanski (44) who in a space of 24 hours registered an average of 14 hours of sleep, distributed over 10 periods, which were longer and more frequent during the day than during the night—was in this way found to have disappeared completely, the rats being awake whenever they were observed. The animals showed a normal interest in their environment. Their general condition was excellent at first and they spontaneously took food and drink. Soon, however, their state deteriorated, which is not surprising considering the large amount of sleep to which the rat is accustomed. After a period of 24 hours the sleepless rats usually began to show symptoms of fatigue. They did not eat or drink of their own accord and their interest in the surroundings decreased. Symptoms of sham rage, if present, persisted. In spite of the fatigue and even of the succeeding exhaustion, during which the gait became unsteady, sleep was not forthcoming, the opened eyes and the spontaneous

activity proving that the animals were awake. After a period averaging three days the exhausted animals fell into a state of coma which soon ended in death. A return of the sleeping capacity was never observed in any of the animals.

We did not observe hypothermia in sleepless rats, nor did these animals develop purulent infections of mucous membranes. In a previous section we incidentally mentioned the disappearance of characteristic periods of sleep in drowsy animals. In these cases both the waking and the sleeping capacity seemed to have been disturbed by lesions, situated in the hypothalamic region between the mammillary bodies and a transverse plane about two

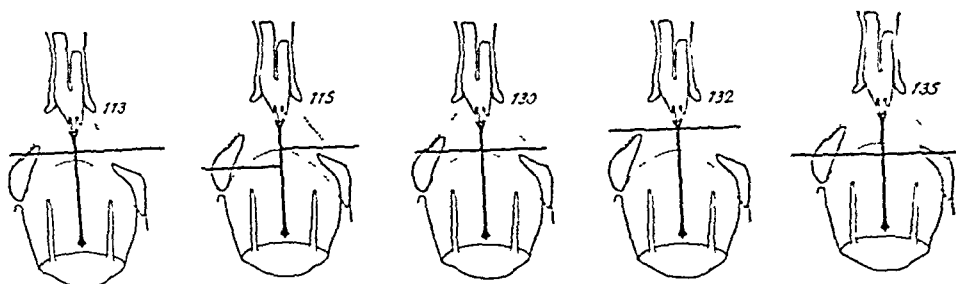


Table 4. Anatomical findings in five rats exhibiting sleeplessness. See Fig. 5.

millimetres in front of these cell groups. The animals referred to in this section, however, showed a loss of the sleeping capacity which was not complicated by any apparent disturbance of the function of waking. In all these cases of uncomplicated asomnia the lesions were found to be situated in the rostral half of the hypothalamus, in contrast to the cases

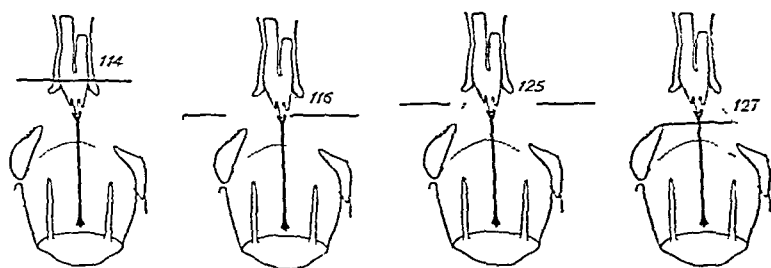


Table 5. Anatomical findings in four rats which did not develop sleeplessness. See Fig. 5.

of sleep or somnolence in which the lesions regularly occupied the caudal half of this part of the brainstem. The border between the two regions lies approximately 2 mm. in front of the mammillary bodies and roughly separates the infundibular and mammillary regions from the more rostrally situated suprachiasmatic and preoptic regions.

Here, also, a long series of verbal descriptions has been omitted. Instead a table is given (Table 4), in which the lesions found in each case are marked in a simple diagram. It will be noticed that in all cases complete bilateral transections were found on various levels in the rostral half of the hypothalamus.

(b) In a second group of animals lesions were found in approximately the same region of the brain. These rats, however, did not develop any apparent disturbance of the sleep-waking rhythm. Four cases of this group are given in Table 5. In one of the animals (rat 114) a transverse section was found in the septal region, slightly rostral to the lamina terminalis. In another rat (no 125) the paramedian region of the brain, including both hypothalami, had been left intact, while in rat 116 only one of both hypothalami had been

cut across. The latter case indicates that the function of sleeping, like that of waking, can be sustained by one hypothalamus.

(c) We observed only one rat in which the operation resulted in a partial loss of the sleeping capacity. This animal (rat 149, Fig. 9) was sleepless during the first postoperative day, but after that time short periods of sleep occurred with highly irregular intervals. The animal's condition failed only slowly; it could be kept alive for nine days.

The lesions found in this rat are in a sense the reverse of those encountered in rat 127 (Table 5), in which the sleep-waking rhythm was not affected by the operation. Whereas in the latter animal only the medial part of the

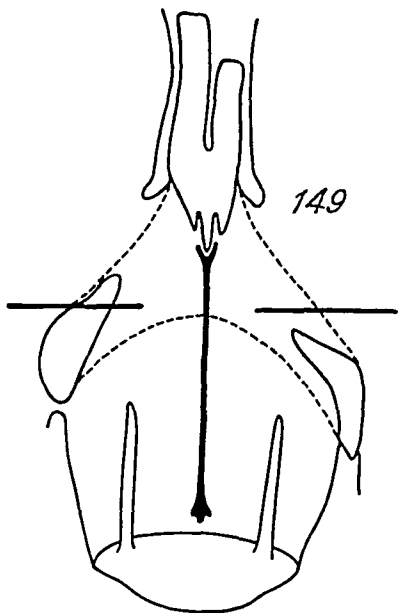


FIG. 9. Lesions in rat 149. Only the lateral area of the suprachiasmatic part of the hypothalamus has been interrupted on both sides.

hypothalamus has been transected, the lesions had been confined to the lateral hypothalamic area in rat 149. Comparison of both cases indicates that the importance of the lateral area of the hypothalamus for the sleeping capacity exceeds that of the inner areas, the latter not being without any significance for this function. It will be noticed that a similar conclusion was reached with relation to the waking capacity. Evidently the rostral half of the hypothalamus, roughly conforming to the suprachiasmatic and preoptic areas, is the site of a nervous structure which is of specific importance for the capacity of sleeping. In the following sections this structure will be referred to as "sleep centre."

This conclusion is in agreement with the conception of von Economo (11) who likewise postulated a rostral hypothalamic centre, to be regarded as the effector of sleep, as proved by the appearance of sleeplessness after its exclusion. In the contradictory opinion of Ranson and Magoun (39), this sleeplessness would not

result from destruction of any part of the hypothalamus but from an irritating influence exerted by the inflammation on the adjacent waking centre. Our results, however, do not produce much evidence in support of the latter view. If the sharp transections which we inflicted to the hypothalamus really caused insomnia only by way of irritation of the waking centre, a unilateral lesion of the rostral part of the hypothalamus would already be sufficient to evoke this phenomenon because one hypothalamus is able to produce diffuse and eventually bilateral reactions on stimulation. As, on the contrary, unilateral lesions fail to cause any appreciable change in the alternation of wake and sleep, we are inclined to ascribe the insomnia to the exclusion of a certain function rather than to irritation of the waking centre.

*Action of the sleep centre*

The question which was discussed in a previous section with regard to the action of the waking centre also applies to the sleep centre: in what way does it act? For the time being we shall confine this problem to the cerebral manifestations of sleep.

The occurrence of somnolence after exclusion of the waking centre is open to two different interpretations. In the first place, it would seem possible that it results from an unopposed direct action of the sleep centre—the only part of the regulating apparatus left intact—on the cortex. As pointed out in the introduction, this theory was advanced by von Economo. Another possibility is an inhibitory action of the sleep centre on the waking centre, and in this case sleep would result from inactivity of the latter, a concept which fits into the theory of Ranson and his co-workers. If sleep is really brought about by an inhibitory action of the sleep centre on the cortex, the exclusion of this centre would tend to abolish or decrease the somnolence which follows on exclusion of the waking centre. If, on the other hand, this somnolence is essentially the result of the abolition of a certain stimulatory action exerted on the cortex by the waking centre, it would not be affected by exclusion of the sleep centre.

In the preceding sections evidence was presented that exclusion of the waking centre can be obtained by transection of the mammillary part of the hypothalamus, whereas the sleep centre seems to be put out of action by a similar lesion in the suprachiasmatic region. The above-mentioned problem can thus be put in a more practical way as follows: is the somnolence which follows on transection of the mammillary region affected by similar lesion in the suprachiasmatic region?

The experiment which can answer this question would seem to be a simple one. Unfortunately, however, all operations in which a suprachiasmatic lesion was inflicted to a rat previously made somnolent by operation ended in the animal's death during or shortly after the operation. It will be remembered that transections of the mammillary region are not, as a rule, survived for more than 4–8 days, and consequently the lapse of time between the two operations is of necessity too short to allow of sufficient recovery, which explains the unfavourable outcome of this experiment. We were therefore compelled to simplify our method, which could only be attained by performing both operations in one session. With this procedure transverse lesions were inflicted to the mammillary and to the suprachiasmatic region simultaneously. It is clear that the outcome of this experiment depended on the degree in which the somnolence resulted from active inhibition of the cortex by the sleep centre. If the additional suprachiasmatic lesion would fail to produce any differences between these rats and those in which only mammillary lesions were present, it would be plausible to exclude any direct action of the sleep centre on the cortex and other major parts of the nervous system. In this case the sleep centre might be supposed to exert its

depressing action on the cortex only via inhibition of the waking centre.

The results of some of our experiments will now be described. In rat 138 the operation resulted in a typical condition of sleep. After waking the animal, which was easily done by pinching its tail, it yawned and made stretching movements, and was soon asleep again. In all respects its behaviour resembled that of the animals shown in Table 1. This condition remained unaltered for seven days, after which the animal unexpectedly died. Autopsy revealed a complete transection of the hypothalamus on a level with the middle of the optic chiasma, and a similar lesion at the rostral border of the mammillary body (Fig. 10). It would appear from this experiment that a transection of the suprachiasmatic region, which in itself is able to cause sleeplessness, fails to interfere with the somnolence resulting from lesion of the mammillary region. Similar observations were made in rat 151, in which identical lesions were found.

*Rat 136.* This animal did not develop a complete condition of sleep. Instead it exhibited a constant drowsiness, from which it could easily be roused and which persisted up to its death four days later. In this rat also transverse lesions were found, one in the suprachiasmatic and one in the mammillary region (Fig. 11). The latter lesion, however, was incomplete, leaving a small part of the right lateral hypothalamic area intact, and, as was mentioned in the discussion of the somnolent animals, incomplete transections of the mammillary region may cause drowsiness. The suprachiasmatic lesion had apparently failed to prevent this decrease of the waking capacity.

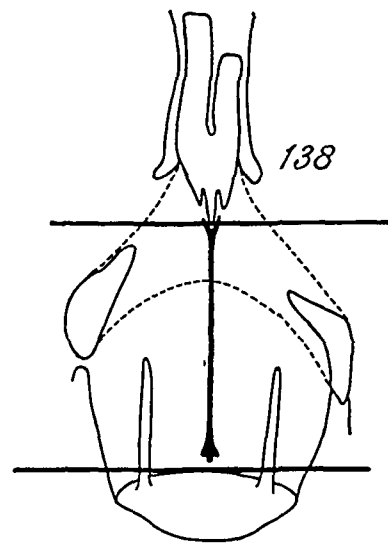


FIG. 10. Hypothalamic lesions in rat 138.

*Rat 140.* During the first two postoperative (days this animal displayed a heavy somnolence which in the following period of ten days gradually declined. On the thirteenth day after the operation the animal was completely awake. There were, however, no normal periods of sleep, so that a condition of sleeplessness developed which was comparable to that of the animals of Table 4, and which resulted in death two days later. Two complete transverse lesions of the hypothalamus were found on autopsy. One was situated in the suprachiasmatic region, the other lay about 1 mm. in front of the mammillary bodies (Fig. 12). This case is instructive in various respects. It has already been mentioned that a partial return of the capacity of waking was observed in

most of the lethargic animals, but that this return was invariably interrupted by the animal's death. In rat 140, however, the period of survival was longer than usual and, for the first time in this experimental series, permitted us to observe a complete return of the capacity of maintaining the waking state. This observation seems to indicate that the function of waking may completely recover from the disorders which follow on lesions of the infundibular region. We do not see any essential difference between this case and those of the previously described group of somnolent rats, and we are disinclined to ascribe the complete return of its capacity of waking to factors other than its longer survival. In our opinion this case offers a confirmation of the conclusion suggested by our findings in rats 138, 151 and 136, *viz.*, that suprachiasmatic lesions are unable to prevent the results of lesions in the mammillary region. It seems that the results of exclusion of the sleep centre depend on the

condition of the waking centre, and that it causes sleeplessness only if the latter is intact. If the waking centre is simultaneously put out of action, no sleeplessness results; instead a condition of sleep develops. These facts seem to exclude any direct action of the sleep centre on the cortex and other major nervous structures. It is therefore highly probable that the sleep centre may cause sleep only by inhibition of the waking centre.

It is striking that the survival period of animals in which both transections had been made was longer than that of rats in which only a supra-chiasmatic lesion was present. In the first case sleeplessness either does not develop at all, or (rat 140) does not immediately develop; in the second group it does. Apparently the vital importance of sleeping is such that even

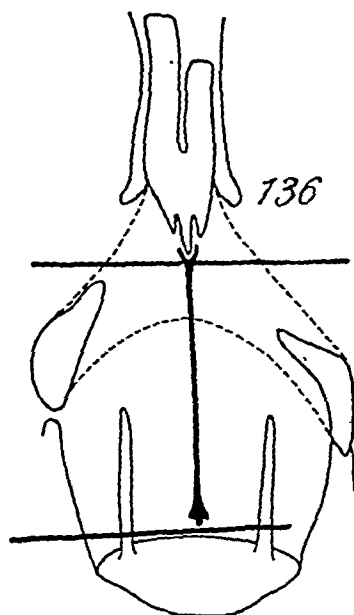


FIG. 11. Hypothalamic lesions in rat 136.

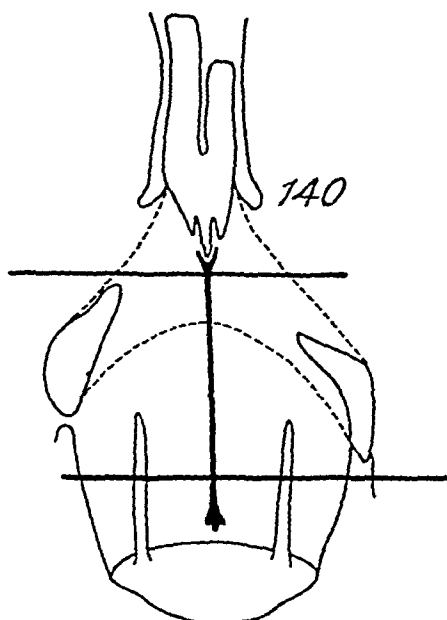


FIG. 12. Hypothalamic lesions in rat 140.

the gross suppression of sleeplessness by means of a second transection of the hypothalamus is able to prolong life.

A large number of fibres which are known to connect the supra-chiasmatic with the mammillary region might account for the transmission of impulses from the sleep centre to the waking centre. It would seem from our experiments that these impulses are chiefly conducted through the lateral hypothalamic area, *i.e.*, through the medial forebrain bundle. In one of the preceding sections the possibility was discussed that the impulses from the waking centre are carried in the opposite direction by the same bundle. It thus seems possible that the medial forebrain bundle is serving a double purpose in the regulation of sleep. Our concept is illustrated by Figure 13 which takes into consideration the facts that (i) a transverse lesion on the

level of arrow 3 (the suprachiasmatic region) causes sleeplessness; (ii) that a similar lesion in the infundibular region (arrow 2) results in drowsiness combined with fewer periods of normal sleep; while (iii) a transection of the mammillary region (arrow 1) is followed by a complete loss of the capacity of maintaining the waking state, which masks the simultaneous exclusion of the sleep centre.

If Hess was right when claiming that a stimulation of the diencephalon with weak galvanic currents may cause sleep, it is possible that he observed the result of direct or indirect activation of the sleep centre. It should, however, again be stressed that it is not yet clear how Hess' findings should be interpreted.

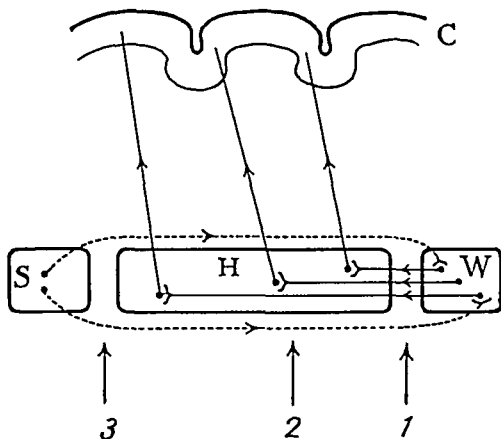


FIG. 13. Diagrammatic representation of the functional relationship between sleep centre and waking centre. Compare with Fig. 7. Fibres carrying "sleep stimuli" have been indicated by interrupted lines. C., cortex cerebri; H., lateral hypothalamic area; S., sleep centre; W., waking centre.

Lastly, von Economo's observation that an initial sleeplessness of patients suffering from epidemic encephalitis usually tends to decline and is gradually replaced by somnolence can be explained on the lines of our concept. Probably the sleeplessness results from inflammation of the sleep centre, and the transition to somnolence seems to indicate a spreading of the process to more caudal hypothalamic parts, finally involving the area of the waking centre. Here, as well as in our experiments, simultaneous exclusion of both centres would result in somnolence. As far as we know, a reverse development of the disease has never been recorded,

and this seems to offer a support for our conception that it is the condition of the waking centre which, as far as the regulation of sleep is concerned, determines the manifestations of inflammatory processes of the hypothalamus.

Our point of view with regard to the difference in opinion between the schools of von Economo and Ranson may be summarized as follows. We agree with von Economo that a certain structure in the rostral part of the hypothalamus can be supposed to be of specific importance for the capacity of sleeping. We do not, however, as von Economo did, believe that this structure may cause sleep by active inhibition of the cortex and other parts of the brain. In this respect we fully agree with the American school that sleep results from functional exclusion of the waking centre. Whereas Ranson and his collaborators hold that periods of sleep are caused by more or less intrinsic periodic decreases in activity of the waking centre, we are in-

clined to attribute these decreases to the inhibitory influence of a sleep centre.

*Hypothalamic localisation of ortho- and parasympathetic centres  
and their relation to sleep regulation*

The localisation of the centres involved in the regulation of sleep suggests a certain antagonism between rostral and caudal part of the hypothalamus. It is interesting to note that certain analogies with the hypothalamic representation of other functions seem to exist. For this purpose we shall turn our attention to the part which is played by the hypothalamus in the regulation of the autonomic balance.

Nearly all investigations of this problem date from the last forty years. After Winkler's observation (47) that stimulation of the hypothalamus of cats gives rise to a production of sweat on the animal's footpads, Karplus and Kreidl (29) started an extensive investigation of the relation between "Gehirn und Sympathicus," which led them to the assumption of an orthosympathetic centre in the lateral hypothalamic area, close to the medial side of the subthalamic nucleus of Luys. In later years this conception received support from a number of investigators of whom Beattie *et al.* (2), Ectors (13), Crouch and Elliott (10), Morison and Rioch (33) and Ranson, Kabat and Magoun (39) should be mentioned. From the studies of Ranson and his co-workers it would appear that the orthosympathetic area is chiefly confined to the infundibular and mammillary regions. Stimulation of these areas generally results in a rise of blood pressure, an increase in rate and depth of respiration, and pupillary dilatation. The same symptoms appear on stimulation of points in the midbrain tegmentum, which suggests an extension of the sympathetic area into the mesencephalon.

Concerning the existence of a parasympathetic hypothalamic centre divergent opinions exist. Positive findings have been recorded by Beattie (2) and Beattie and Sheehan (4), who observed a number of parasympathetic phenomena on stimulation of the rostral part of the hypothalamus, *viz.*, augmentation of intestinal peristalsis, gastric secretion, decrease of heart rate and of blood pressure, and pupillary constriction. Like Ranson *et al.*, they were able to produce sympathetic symptoms by stimulating the caudal part of the hypothalamus. On the basis of these findings they divide the hypothalamus into a rostral parasympathetic and a caudal sympathetic part.

Later studies offered little confirmation of their concept. Ectors, Brookens and Gerard (14), and also Crouch and Elliott (10), were unable to evoke other than sympathetic phenomena from both parts of the hypothalamus. Morison and Rioch (33) observed a few parasympathetic phenomena only on stimulation of the rostral pole of the amygdaloid nucleus, the basal part of the septum and the lamina terminalis, *viz.*, a relaxation of the nictitating membrane and a decrease of blood pressure. They were led to the conclusion that these parts of the brain contain a mechanism for inhibition of sympathetic activity. In their admirable investigation of the problem, Ranson, Kabat and Magoun (38) observed some parasympathetic phenomena on stimulation of an area which, although it belongs to the surroundings of the third ventricle, is not regarded as a part of the hypothalamus



by Ranson, *i.e.*, the preoptic region. Stimulation of this area resulted in contraction of the bladder and in decrease of blood pressure and of rate and depth of respiration, but was never followed by miosis and intestinal peristalsis, as claimed by Beattie. The depressor reactions could also be elicited by stimulation of several parts of the medial hemispheric wall such as the septum and the genual gyrus. By their results Ranson *et al.* are led to the conclusion that the caudal hypothalamic region contains a complete sympathetic centre, but although some parasympathetic functions seem to be localised in the preoptic region and in adjacent parts of the medial wall of the hemisphere, they are disinclined to regard this area as a complete parasympathetic centre. They further accept the existence of a separate centre in the septal region for contraction of the bladder.

The appearance of parasympathetic phenomena on stimulation of the relevant hypothalamic area is open to two different interpretations. In the first place, one could imagine a conduction of its stimuli to lower parasympathetic centres and from there to the end-organs concerned. Although this mechanism may be involved, it is doubtful whether it offers sufficient explanation for the results of anterior hypothalamic stimulation. There would be little doubt if the parasympathetic picture were more or less complete, but, as pointed out above, intestinal peristalsis, for instance, is lacking. Apart from the contraction of the bladder which seems to have a separate representation in the endbrain, the main results of stimulation of the parasympathetic hypothalamic area are depressor in nature, and this suggests an inhibitory action on the sympathetic centre in the caudal hypothalamic region. It does not seem improbable that the parasympathetic area of the forebrain is essentially a regulator of sympathetic activity. It will be remembered that a similar view is held by Morison and Rioch.

When comparing this concept with that displayed in previous sections, a conspicuous analogy between the regulations of sleep and of autonomic balance is met with. In both functions the rostral part of the hypothalamus seems to exert a depressing action on the caudal part. By this striking congruity in hypothalamic representation one is led to ask what relation exists between the cyclic changes of activity and the autonomic balance. It has already been mentioned that the waking centre and the sympathetic hypothalamic centre are topographically related and perhaps even identical. Moreover, it has long been recognized that the transition from the waking to the sleeping state corresponds to a shift of the autonomic balance from the sympathetic to the parasympathetic side, while the process of awakening is accompanied by a counterchange of autonomic activity. Hess (22) even claims that sleep results from a preponderance of the parasympathetic system, and that the waking state is established by an increase of sympathetic activity. The enhancing influence of adrenalin and related drugs (benzedrine, etc.) on consciousness is in tune with his conception that the waking state is dominated by the sympathetic system, but although it is certain that the autonomic balance lies on the parasympathetic side during sleep, there is little reason to regard sleep as a result of parasympathetic preponderance, because those drugs which are able to imitate parasympathetic activity (acetylcholine and its relatives) fail to produce sleep. Ranson and Magoun (39) are therefore inclined to regard the preponderance of the parasympathetic system during sleep as a result of a decrease of sympathetic activity. This conception is in accordance with our observation that sleep results from inhibition of the caudal hypothalamic area, which contains the highest sympathetic centre.

By way of summary it seems possible to accept a certain topographical and functional congruity between the centres involved in the regulation of sleep and those subserving the regulation of the autonomic balance. Probably both functions are manifestations of one diencephalic mechanism. So far we have dealt only with cerebral symptoms of the cyclic changes of activity. All phenomena belonging to the somatic sphere have been omitted in order to avoid a premature complication of our display, all efforts to explain the somatic manifestations of sleep meeting with considerable difficulties.

It is highly probable that many functions of lower parts of the central nervous system are actively inhibited during sleep. According to Magnus

(17, 18), for instance, the maintenance of the recumbent position during sleep can be explained only by accepting an inhibition of the red nucleus. An important indication of active inhibition of lower centres was recorded by Tarchanow (cited from 35), who demonstrated that the normal inhibition of spinal reflexes during sleep was missing in the distal part of the spinal cord after complete transection in the thoracic region. Apparently the normal suppression of the spinal cord during sleep had been abolished for the segments below the level of the lesion. One is led to ask in which part of the central nervous system this inhibiting mechanism is localised.

According to Gamper (17, 18), no intrinsic changes in intensity are discernible in the decerebrate rigidity which appears after transection below the red nucleus. This would mean that the cyclic changes in muscle tone, which are characteristic of the sleep-waking rhythm, are abolished by decerebration. Gamper arrives at the following conclusion: "Wir können also in den Abschnitten des Zentralorgans, die distal vom Gebiete des roten Kerns liegen, keine den phasischen Ablauf der Lebensvorgänge regulierende Zentralstelle vermuten."

In a child in which the endbrain had not been developed, Gamper observed irregular periods of sleep. On autopsy the hypothalamus turned out to be poorly developed, but the transitional zone between fore- and mid-brain and the whole neural tube caudal to this region were fairly normal. From these facts Gamper concludes that the nervous apparatus for the regulation of sleep must be localised in the rostral part of the midbrain or in the hypothalamus.

It would seem reasonable to suppose that the inhibition of the lower parts of the brain during sleep is a function of the sleep centre in the rostral part of the hypothalamus. Many difficulties, however, arise on trying to answer the question as to along what ways its impulses descend. There is reason to believe that the influence of the sleep centre is not mediated to lower centres by one uninterrupted pathway, because certain lesions in a lower part of the brainstem, *viz.*, the mammillary region, instead of preventing all somatic manifestations of sleep, result in a typical condition of sleep. Clinical observations indicate that the regulating mechanisms of somatic and cerebral sleep are to a certain extent separate. It is, for instance, a well known fact that soldiers on a long march may finally show unmistakable symptoms of cerebral sleep while walking perfectly, which suggests a certain mutual independence of the centres for somatic and cerebral sleep. Cataplexy is per-

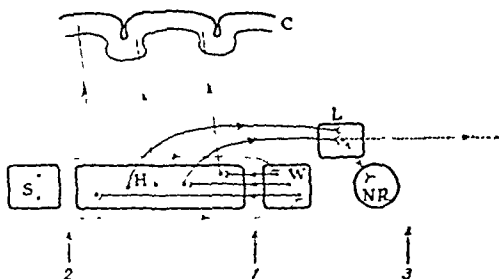


FIG. 14. Diagrammatic illustration of the mechanism of sleep, as proposed in text. C., cortex cerebri; H., lateral hypothalamic area; L., "centre for body sleep"; N.R., nucleus ruber; S., sleep centre; W., waking centre. Fibres conducting "waking" stimuli are indicated by solid lines; broken lines represent fibres involved in the production of sleep.

haps an opposite condition. Normally, however, both manifestations of sleep make a simultaneous appearance, which indicates the existence of a superior correlative mechanism. So long as exact data are lacking, no statements can be made concerning the pattern on which the complete phenomenon of sleep is effected. A mechanism which at present seems conceivable is diagrammatically illustrated by Figure 14. We have accepted the existence of a separate centre for somatic sleep (L), situated caudal to the waking centre and inhibited by it via the lateral hypothalamic area during the waking condition. Under normal conditions it would be able to exert its depressing influence only on lower centres (exemplified in our diagram by the red nucleus) after being freed from the action of the waking centre. As discussed in a previous section, this liberation may result from activity of the sleep centre S.

Our diagram offers an explanation of the facts: (i) that a transection slightly caudal to the sleep centre (arrow 2) causes sleeplessness; (ii) that a similar lesion on the level of arrow 1 (the mammillary region) gives rise to a complete condition of sleep; (iii) that transections below the level of the red nucleus (decerebration arrow 3) are followed by a condition in which all somatic symptoms of sleep are lacking.

No experimental data on dissociation of cerebral and somatic sleep can be found in literature. If our conception of a spatial separation of the sleep centre L from other parts of the regulating apparatus is correct, it would not seem impossible that certain lesions are able to produce isolated disturbances in the regulation of either cerebral or somatic sleep.

### DISCUSSION

In the foregoing account the expressions "waking centre" and "sleep centre" have been repeatedly used. In order to avoid misunderstanding it should be emphasized that the existence is uncertain of smaller or larger groups of specific cells subserving the regulation of sleep only. The function of each of the separate hypothalamic cell groups—with the exception of the supraoptic nucleus, of which the essential role in the regulation of the salt and water balance has been demonstrated by Fisher *et al.* and others—is still unknown. So long as more exact data are lacking, we are able to state only that some structure in the caudal half of the hypothalamus and in the adjacent part of the tegmentum mesencephali is of specific importance for the maintenance of the waking state, while the preoptic region is likely to contain a structure subserving the function of sleeping. For the sake of brevity these structures may be referred to as "waking centre" and "sleep centre" respectively, but these terms should not give the impression that anything more than what has been stated above can be said concerning them. For instance, the possibility that the regulation of sleep is only one of multiple functions of one single nervous apparatus cannot be excluded since there seems to exist a topographical identity between the hypothalamic regions involved in the regulation of sleep and those regulating the autonomic

balance. The current conception of "centres" has been criticised by Bethe (5), who, by the extreme plasticity of the nervous system of invertebrates and of the more centralized nervous system of higher animals, was led to reject the thought that nervous functions are carried out by specific centres. Nevertheless, one is forced to the conclusion that the nervous structures normally involved in the regulation of sleep occupy a small area, only lesions of a circumscribed location being able to interfere with this regulation. Therefore, if the above restrictions are taken into consideration, the term "centre" would not seem to be inappropriate for indicating these structures.

In the experiments of Ranson *et al.*, as well as in ours, "plasticity" was observed in the function of maintaining the waking state. It is still uncertain whether such recoveries from injuries of nervous tissue must be ascribed to compensatory activity of secondary centres already involved in the function under consideration—a conception which is well illustrated by Ch. Foix's term "automatisme étagé" (45)—or to the formation of a new regulating apparatus in a part of the brain which has afferent and efferent connections similar to those of the destroyed area but is situated outside the shortest route in normal animals, or to some other compensating mechanism. It is a striking fact that no return of the capacity of sleeping could be observed in any of our sleepless rats, not even in rat 140 who survived for 13 days.

Cyclic changes in activity were observed in decorticated dogs by Goltz (20) and Rothmann (41), and by Gamper (17, 18) in an acerebral child. Although it is well known that cortical processes have an important influence on the sleep-waking rhythm, we are justified in denying the existence of a dominating autonomic cortical mechanism for the regulation of sleep. It should be stressed that in animals in which a maximal decrease of the waking capacity had been obtained a normal waking condition could still be evoked by sufficiently strong external stimuli. Only the capacity of *maintaining* this condition had apparently been lost. The concepts of sleep being caused by abolition of sensory stimuli seem to apply to these cases only. It is probable that the waking centre endows the cerebral cortex with a power of maintaining a certain functional "tone" in the absence of external stimuli, and that only sensory stimulation of sufficient strength is able to prevent the onset of sleep when the waking centre is reduced to inactivity.

It scarcely needs to be emphasized that our knowledge concerning the course of the impulses from the waking centre to the cerebral cortex is incomplete. If our concept of an ascending flow of these impulses through the medial forebrain bundle is correct, what connections may account for their further transmission is still obscure. It seems improbable that the thalamus is involved in this transmission to any important extent, which, in conjunction with von Economo's observations on the sensibility of his encephalitic patients, renders it highly probable that the waking centre does not essentially act via sensibility. Of what its influence consists is a question open to further investigation.

Our conception concerning the existence of a sleep centre is based solely

on the observation that no periods of sleep could be observed after complete transections in the suprachiasmatic region of the hypothalamus. Kabat, Anson, Magoun and Ranson (28), however, do not mention sleep among the results of stimulation of this region in waking cats, and Ranson *et al.* did not observe sleeplessness after its partial destruction (39). The disagreement between our findings and those of Ranson *et al.* may lie in the difference in techniques. It seems necessary to repeat our experiments in other animals in order to ascertain whether sleeplessness is a general result of anterior hypothalamic transection.

### SUMMARY

1. In the rat complete bilateral transection of the hypothalamus, irrespective of location, interferes with the normal regulation of sleep.

2. The location of lesions which cause disturbances of the function of waking indicates the existence of a structure in the caudal hypothalamic region and in the adjacent part of the midbrain tegmentum, which is of specific importance for the capacity of maintaining the waking state during the absence of external stimuli ("waking centre").

3. There is reason to accept a structure in the preoptic region, which is of specific importance for the capacity of sleeping ("sleep centre").

4. Evidence is offered that sleep is caused by an inhibitory action of the sleep centre on the waking centre.

5. The lateral hypothalamic area seems to be of more importance for the regulation of sleep and waking than the inner areas. It seems probable that the medial forebrain bundle, which occupies this area, is implicated in the transmission of impulses determining the sleep-waking rhythm.

6. The hypothalamic centres involved in the regulation of sleep are topographically identical with those determining the autonomic balance.

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# FREQUENCY OF CENTRIPETAL STIMULATION IN INHIBITION AND FACILITATION OF THE KNEE-JERK

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MANY INVESTIGATORS have studied the reflex effects produced by a single volley or brief tetanic volleys upon a background of excitatory reflexes (1). Some authors have made scattered comments upon the relation of inhibition to the frequency of afferent stimulation (3, 11). The data in the literature, however, do not warrant conclusions about the relation of the intensity of the inhibitory process to the frequency of afferent stimulation. The experiments reported in this paper were undertaken with the object of investigating this relation. Inhibition of the knee-jerk was chosen because it is one of the simplest (*i.e.*, involving the fewest neurons) inhibitory reflexes known (8, 9). Experimental conditions were simplified by using the acute spinal cat. It soon became obvious that stimulation of any nerve in the hind legs of a spinal cat produces mixed effects, excitatory and inhibitory, upon the motoneurons innervating the quadriceps muscle. The present report is a description of the influence of frequency and duration of the centripetal stimulation in facilitating and inhibiting the knee-jerk and in producing other reflex activity of this muscle. The data presented permit an analysis of the role of the frequency of afferent stimulation in determining the intensity of the inhibitory and excitatory processes.

## METHODS

In etherized cats, the spinal cord was completely sectioned in the lower thoracic region (T8-L1), most frequently between T11 and T12. The cat was then decerebrated by blunt section through a trephine hole and the ether inhalation discontinued. Experimental observations were not begun until an hour or more after the cessation of the ether inhalation. The left leg was immobilized by drills inserted into the ends of the femur. The left quadriceps muscle was partially isolated from the neighboring muscles and attached by its tendon to a lever so that the muscle pulled against a rubber band. The kymograph records reveal mainly changes in tension of the muscle. The magnification was 12- to 15-fold. The exposed portion of the muscle was covered with vaseline to prevent drying.

A motor-driven apparatus delivered taps to the lever connected to the quadriceps muscle, thus eliciting knee-jerks. In the experiments to be described below, taps were delivered at a rate of one every four seconds or more slowly, unless otherwise stated. The taps were slightly supramaximal with respect to the knee-jerk. A tap was considered maximal if it produced a maximal knee-jerk. The left hamstring and sciatic nerves were routinely cut, except in those experiments in which the peroneal or the superficial branch of the

<sup>1</sup> The experiments reported in this paper were performed while the author held the Elizabeth Avery Colton Fellowship of the American Association of University Women for 1943-1944. These experiments were reported in a thesis, *Inhibition of spinal and bulbar reflexes in mammals*, which was accepted in partial fulfillment of the requirements for the Ph.D. degree at Radcliffe College in June, 1944.

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peroneal was stimulated. Shielded silver-wire electrodes were placed under the nerves which were to be stimulated. The peripheral ends of these nerves were either cut or crushed. The electrical stimuli consisted of condenser discharges through a thyatron. These stimuli were passed through a transformer before application to the nerve. Stimuli were considered maximal with respect to inhibition if increasing the intensity of the stimuli did not further increase the inhibition.

## RESULTS

*Background of inhibition.* Records of knee-jerks were obtained in 82 spinal cats. The regularity of the knee-jerks determined the suitability of a preparation for demonstrating inhibition, the absolute amplitude being relatively unimportant. When elicited at a rate of one tap every four seconds, or more slowly, knee-jerks were judged to be satisfactory if the variation in amplitude of the uninhibited knee-jerks was not more than 10 per cent of the average amplitude. About 75 per cent of the spinal cats had satisfactory knee-jerks. In 16 per cent of the preparations, the knee-jerks varied in amplitude more than 10 per cent, but nevertheless maintained sufficient regularity to serve as a background for inhibition. Nine per cent (seven cats) had small, highly irregular knee-jerks which were not satisfactory for studying inhibition.

The taps were given continuously in experiments lasting eight or more hours without a noticeable impairment of the knee-jerks, when judged with respect to both their size and regularity. Control experiments showed that the knee-jerk and its inhibition were only slightly affected by hypercapnia, hyperventilation, and by changes in body temperature (from 30°C. to 42°C.). Hypercapnia was produced by letting the cat breathe for about 15 minutes a mixture of air and oxygen containing 7.5 per cent CO<sub>2</sub> from a large spirometer. The effect of hyperventilation was studied by administering artificial respiration for about 15 minutes with a stroke volume of from two to four times the normal tidal air. The body temperature, which was recorded rectally, was lowered by placing the forelegs in a pan of cold water and letting a fan blow on the animal. The body temperature was raised by a heating pad which was placed under only that portion of the body which was cephalad to the spinal section. These experiments support the conclusion of King, Garrey, and Bryan (7): "The effects of acidosis, alkalosis, and anoxia within physiological limits play but a small role in accounting for the variability in spinal reflex responses . . ." (see also 12). Sudden large changes in blood pressure did not affect the amplitude and regularity of the knee-jerks. The stability of the preparation made possible the numerous long experiments required in a study of the role of frequency of centripetal stimulation in inhibition and facilitation of the knee-jerk.

*Nerves used for inhibiting knee-jerks.* Marked inhibition of the knee-jerk was obtained by stimulating one of the following ipsilateral nerves: the hamstring nerve, a branch of the hamstring nerve, the sciatic (peroneal-popliteal) nerve, the peroneal, and the superficial branch of the peroneal nerve. These experiments confirmed the conclusion of previous investigators (1) that stimulation of the hamstring nerve is slightly more effective in producing inhibition of the knee-jerk than stimulation of the sciatic. Aside from this distinction between these two nerves, differences in the inhibitory effect upon the knee-jerk of stimulation of ipsilateral nerves appeared to be related mainly to the number of fibers contained in the nerve. Maximal stimulation of a large nerve such as the sciatic or hamstring produced more nearly complete inhibition, when averaged over a period of several minutes, than maximal stimulation of a small nerve. Throughout this report, a

"large" nerve refers to the sciatic or hamstring nerve; a "small" nerve refers to the superficial branch of the peroneal or to a branch of the hamstring nerve. Stimulation of the ipsilateral saphenous nerve produced no inhibition in one preparation and only slight inhibition in a second preparation.

*Irregularity of inhibited knee-jerks.* The uninhibited knee-jerks were only slightly irregular. This irregularity was markedly increased during periods of incomplete inhibition produced by centripetal stimulation of an ipsilateral nerve-trunk (Figs. 1, 2). There was no rhythm or strict relation of this increased irregularity to the frequency of centripetal stimulation.

*Ipsilateral extensor activity.* In many experiments, a contraction of the quadriceps muscle occurred in response to centripetal stimulation of an ipsilateral nerve-trunk (Fig. 3). The existence of this ipsilateral extensor reflex has been previously reported by T. Graham Brown and Sherrington (4), McCouch *et al.* (10), and others. The knee-jerk is also an ipsilateral extensor reflex. To avoid confusion, in this report the terms "ipsilateral extensor reflex," "ipsilateral extensor activity," and "ipsilateral extensor responses" refer to reflex contractions of the extensor muscle (quadriceps) produced by centripetal stimulation of an ipsilateral nerve-trunk and *not* to the knee-jerk. In about 75 per cent of the preparations, ipsilateral extensor activity was evident for one or more of the frequencies used.

The character of the ipsilateral extensor activity depended upon the frequency of stimulation (curve D in Fig. 9). Although occasionally large

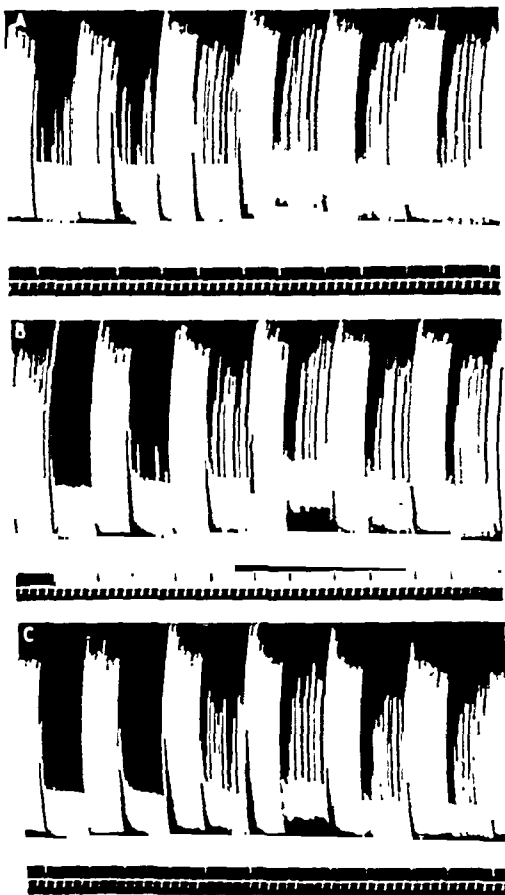


FIG. 1. Inhibition of the knee-jerk produced by centripetal stimulation of the ipsilateral sciatic nerve at different frequencies and intensities. From left to right, the frequencies of stimulation were 120, 60, 30, 15, 7, and 3 per second. A, Weak intensity of stimulation; B, Intensity about 50 per cent greater than in A; C, Supramaximal stimulation. (Intensity about four times that in A. Stimuli were considered maximal with respect to inhibition if increasing the intensity did not further increase the inhibition.) Taps delivered continuously at a rate of one tap every four seconds. Decerebrate spinal cat. Time in one-minute intervals.



FIG. 2. Inhibition of the knee-jerk produced by maximal, centripetal stimulation of the ipsilateral peroneal nerve. From left to right, the frequencies of stimulation are: 120, 60, 30, 15, 8, 4, 2, and 1 per second. Decerebrate spinal cat. Time in one-minute intervals.

at 60/sec. or 120/sec., this activity almost always subsided within 30 seconds at these frequencies, despite continued stimulation. At 15/sec. or 30/sec., the response was more lasting and often slightly larger than at higher frequencies. At frequencies of 8/sec. or 4/sec. ipsilateral extensor activity was absent in about 50 per cent of the preparations. When present, it consisted of a series of small twitch-like responses instead of the reflex tetanus which appeared at frequencies of 15/sec. or greater. The activity did not subside quickly at these frequencies. The ipsilateral extensor activity decreased progressively throughout a long experiment. It was frequently small or absent when the ipsilateral nerve used for stimulation was the hamstring nerve.

*Measurement of inhibition of knee-jerks.* In some experiments a quantitative index of the inhibition of the knee-jerk was desirable. The irregularity of the inhibited knee-jerks and the presence of ipsilateral extensor activity made measurements only approximate. The average height of the inhibited knee-jerks was determined from measurements of the individual responses during a convenient period such as two minutes. The responses to taps which occurred during a marked ipsilateral extensor contraction were disregarded. The average height of the uninhibited knee-jerks was computed from measurements of the 10 or 15 knee-jerks which immediately preceded the application of the inhibitory stimulus. These average heights enabled one to calculate the per cent inhibition.

The inhibition obtained by maximal centripetal stimulation of a *small* ipsilateral nerve at 120/sec. was greater than that obtained at 60/sec. (see next paragraph). Maximal stimulation of a *large* ipsilateral nerve at 60/sec. or 120/sec., however, usually produced 100 per cent inhibition (abolition) of the knee-jerk as long as the stimulation was continued (up to two hours).



FIG. 3. Duration of inhibition of the knee-jerk following the cessation of centripetal maximal stimulation for one second of the ipsilateral sciatic nerve. The frequencies of stimulation were: A, 8/sec.; B, 15/sec.; C, 30/sec.; and D, 60/sec. Taps were delivered at a rate of almost 2/sec. Decerebrate spinal cat. Time in one-second intervals.

See Figs. 1, 2). It is reasonable to infer that with both large and small nerves the intensity of the process underlying inhibition of the knee-jerk is greater at 120/sec. than at 60/sec. When a background of excitatory reflexes is abolished, it can no longer serve as an indicator of the intensity of the inhibitory process.

*Frequency of ipsilateral stimulation.* Correlation of the knee-jerk inhibition with the frequency of centripetal stimulation of an ipsilateral nerve-trunk is presented diagrammatically in curve B of Figure 9. The most marked inhibition was produced by ipsilateral stimulation with frequencies greater than 60/sec. Maximal stimulation of a small nerve produced greater inhibition at 120/sec. than at 60/sec. Sometimes, stimulation of a large nerve produced complete inhibition at a lower stimulus intensity at 120/sec. than at 60/sec. (Fig. 1).

In 38 out of 39 cats in which inhibition at 30/sec. was compared with that at 120/sec., the inhibition (when measured over a period of two or more minutes) was about 25 per cent less at the lower frequency. The difference between per cent inhibition at 30/sec. and at 15/sec. was usually small (Fig. 1); one could not predict at which frequency the inhibition would be greater.

Much more variability in different preparations occurred when the ipsilateral nerve was stimulated at frequencies near 8/sec. than at any other frequency studied. In 22 out of 32 cats in which a comparison was made, inhibition was more nearly complete at 8/sec. than at 30/sec. In two of the other ten cats, inhibition became progressively less marked as the frequency was lowered. Measurements of inhibition at frequencies between 8/sec. and 30/sec. were impossible in another cat because the ipsilateral extensor responses were too large and prolonged. In the remaining seven cats, the inhibition at 8/sec. was approximately equal to that at 15/sec. or 30/sec., when averaged over a period of from two to four minutes. Inhibition at 3/sec. was similar to that at 8/sec. (Fig. 1).

Inhibition at 1/sec. (Fig. 2) was less than 55 per cent complete when averaged over a period of three minutes in seven preparations. In four of these, inhibition at 1/sec. had an average value of 27 per cent.

*Role of intensity of inhibitory stimulus.* In six cats, records of inhibition of the knee-jerk were obtained over a wide frequency range and at intensities varying from threshold to maximal with respect to inhibition (see Fig. 1). Varying the intensity of the stimuli did not change qualitatively the relation of inhibition to the frequency of stimulation.

*Duration of inhibition following brief stimulation.* In nine preparations the duration of inhibition of the knee-jerk was measured following stimulation of a large ipsilateral nerve with a single shock or for one second at frequencies up to 60/sec. Taps were delivered at a rate of 2/sec. After each test there was a three-minute pause during which no taps were delivered.

The curve relating the duration of inhibition to the frequency of stimulation varied considerably in shape in the different preparations (curve C in Fig. 9). The maximum duration, however, always occurred at the highest

frequency studied (60/sec.). It varied from two to eleven seconds in eight of the preparations. In the remaining cat it was less than one second. The duration of inhibition did not increase significantly as the frequency was raised from 1/sec. to 4/sec. From 4/sec. to 60/sec. the duration of inhibition increased steadily with frequency (Fig. 3). It was never longer at 8/sec. than at 15/sec. or 30/sec. The duration after two shocks at an interval of 0.5 second was approximately the same as that reported by Ballif, Fulton and Liddell (1) for a single shock.

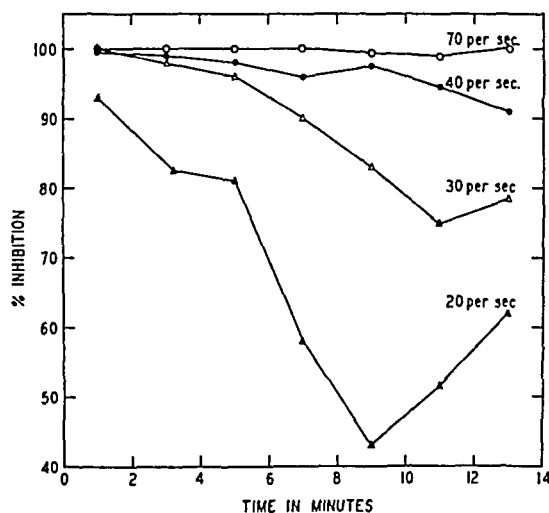


FIG. 4. Impairment of the inhibitory effect upon the knee-jerk of supramaximal (with respect to inhibition), centripetal stimulation of the ipsilateral hamstring nerve at different frequencies. The stimuli were passed through a transformer before application to the nerve as a precaution against polarization at the electrodes. The taps were delivered continuously at a rate of one tap every four seconds. There was an interval of at least five minutes between periods of centripetal stimulation of the hamstring nerve, during which taps were delivered. The points represent the average per cent inhibition obtained in successive two-minute periods of a fourteen-minute period of stimulation. Decerebrate spinal cat.

*Impairment of inhibition.* At frequencies higher than 60/sec. a reversible impairment of inhibition occurred when supramaximal stimuli were applied to a *small* nerve. Even when a *large* nerve was stimulated at a frequency lower than 60/sec., a similar impairment was usually observed. Each curve in Figure 4 represents the average per cent inhibition in successive two-minute periods during 14 minutes of centripetal stimulation of a large ipsilateral nerve at a given frequency. For frequencies between 70/sec. and 20/sec., the rate of impairment of inhibition was greater the lower the frequency.

At frequencies lower than 15/sec. the time required to demonstrate an impairment of inhibition varied in different animals. In some, complete inhibition was maintained for five minutes by stimulation of the ipsilateral nerve at a frequency of 8/sec. (Fig. 2). In others, the inhibition at 8/sec. was more rapidly impaired (Fig. 1). In six preparations an impairment of inhibition occurred more rapidly when the taps were delivered at rates of three or four per second than at the slower rate of one every four seconds (Fig. 5.).

*Facilitation of knee-jerk.* An increased amplitude and greater regularity of the knee-jerks during centripetal stimulation was interpreted as facilitation. The facilitation which results from stimulation of a contralateral nerve was studied in eight preparations. Facilitation increased continuously with

frequency from 1/sec. to 30/sec. It was usually slight when the frequency was lower than 8/sec. In most of the preparations the facilitation of 15/sec. was almost as great as that at 30/sec. At frequencies higher than 30/sec. and up to 120/sec., facilitation was usually the same as that at 30/sec. This relation of facilitation to stimulus frequency is represented by curve C in Figure 9. Facilitation of the knee-jerk outlasted the contralateral extensor responses (Fig. 6). With repeated periods of stimulation of a contralateral nerve, these

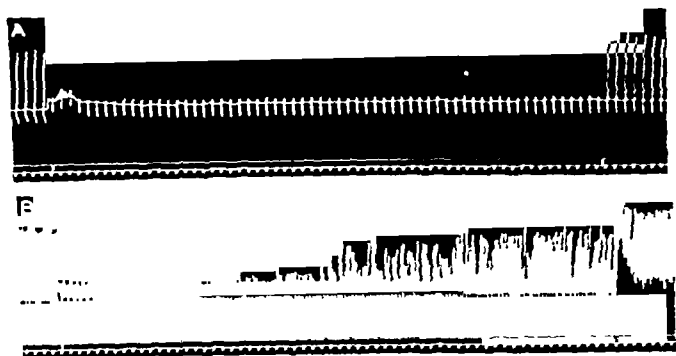


FIG. 5. The influence of the rate of the taps upon the time required to demonstrate an impairment of inhibition of the knee-jerk. The inhibition was produced by supramaximal centripetal stimulation of the ipsilateral sciatic nerve at fifteen per second. A, one tap every six seconds; B, three taps per second. There was a pause of five minutes before each record, during which no taps were delivered. Decerebrate spinal cat. Time in five-second intervals.

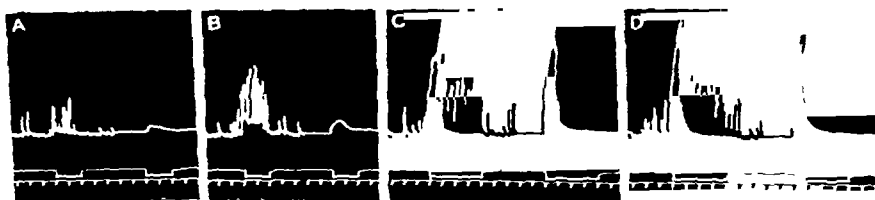


FIG. 6. Facilitation of the knee-jerk. The taps were slightly subliminal for eliciting the normal knee-jerks. Facilitation was produced by stimulation of the contralateral sciatic nerve. The frequencies of stimulation were: A, 4/sec.; B, 8/sec.; C, 15/sec.; D, 30/sec. In each record, the first signal indicates stimulation while taps were being delivered at a rate of one every four seconds. The second signal in each record indicates stimulation at the same intensity and frequency without simultaneous taps. Decerebrate spinal cat. Time in thirty-second intervals.

extensor responses became less prominent. If stimuli were applied simultaneously to a contralateral nerve and an ipsilateral nerve, the extensor response characteristic of the stimulation of the contralateral nerve alone was partially or completely inhibited. The inhibition of the knee-jerk during simultaneous stimulation of the ipsilateral and contralateral nerves was less than that produced by stimulation of the ipsilateral nerve alone.

*Excitatory rebound.* Rebound may be defined as a change in the response of an effector, other than recovery, which follows the cessation of some

afferent stimulation. It may be classified as supraliminal if it automatically follows the cessation of the afferent stimulation. Subliminal rebound can be detected only by the altered response of the effector to some additional afferent stimulus. In these experiments, subliminal rebound was revealed by

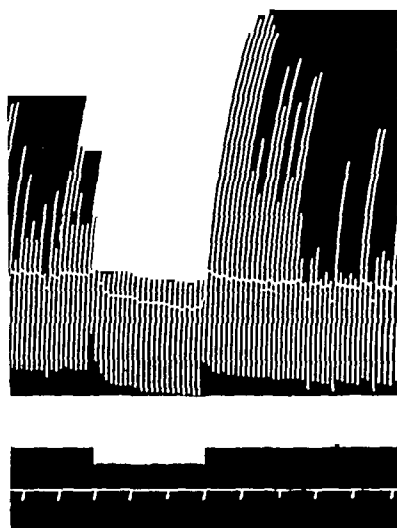


FIG. 7. Excitatory rebound in a cat in which the normal knee-jerks were small and irregular. Centripetal stimulation of two of the three branches of the ipsilateral hamstring nerve is indicated on the middle signal line. Note (i) the inhibition of the knee-jerks during the stimulation, and (ii) the large, regular knee-jerks, following the stimulation, which indicate that subliminal excitatory rebound had occurred. The frequency of stimulation was 120 per second. Decerebrate spinal cat. Time in one-minute intervals.

a change in the character of the knee-jerk (Fig. 7). It was more common than supraliminal rebound. Supraliminal rebound was always accompanied by subliminal rebound which could be detected by delivering taps after the supraliminal rebound had subsided.

Table I and Figure 8 summarize the observations upon the rebound which followed stimulation of an ipsilateral nerve for the relatively long period of from one to five minutes. The conditions were not suitable for

Table I. The excitatory rebound which followed maximal, centripetal stimulation of an ipsilateral nerve-trunk for a period of from one to five minutes. Taps for eliciting knee-jerks were delivered continuously at a rate of one every four seconds or more slowly.

Stimuli per sec.	No. of Cats	Prominence of rebound							
		Absent		Small		Moderate		Marked	
		No. of Cats	Per cent	No. of Cats	Per cent	No. of Cats	Per cent	No. of Cats	Per cent
1	6	5	83	1	17	0	0	0	0
2	4	4	100	0	0	0	0	0	0
4	22	15	70	2	9	3	12	2	9
8	33	18	55	4	12	8	24	3	9
15	38	19	50	13	34	4	10	2	5
30	42	24	57	8	19	5	12	5	11
60	44	27	61	5	11	7	16	5	11
120	36	28	78	3	8	4	11	1	5

distinguishing between supraliminal and subliminal rebound because the taps were being delivered continuously. The Table indicates that rebound rarely followed stimulation at frequencies equal to or lower than 4/sec. The graph in Figure 8 shows that rebound occurred in from 40 to 50 per cent of the preparations at frequencies between 8/sec. and 60/sec.; it was most common after stimulation at 15/sec. (see also curve F in Fig. 9). The rebound was marked after stimulation with frequencies between 8/sec. and 60/sec. in about 10 per cent of the preparations. Marked rebound was slightly less frequent after stimulation of the hamstring nerve than after stimulation of the sciatic nerve. Stimulation of a contralateral nerve sometimes produced excitatory rebound.

After stimulation of an ipsilateral nerve for a period of one second, no excitatory rebound was seen; on the contrary, the long-lasting inhibitory effects represent subliminal inhibitory rebound (p. 321, Fig. 3). In some experiments excitatory rebound became obvious after ipsilateral stimulation for two seconds, and it became marked after stimulation for ten seconds. The influence of the rate of tapping upon excitatory rebound is illustrated in Figure 5. No rebound was evident when the knee-jerks were elicited at a rate of one tap every six seconds. Rebound was marked when the taps were delivered at four per second.

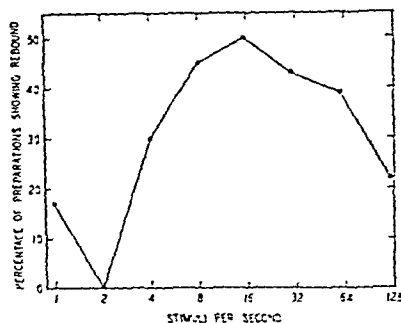


FIG. 8. A graphical summary of the data in Table I.

### DISCUSSION

At a given moment the activity of the anterior horn cells innervating the quadriceps muscle may be considered to be the resultant of the excitatory and inhibitory processes affecting these cells. In the experiments reported here, a periodic predominance of excitatory process (the knee-jerks) was provided in order to be able to detect the changes of the balance between the two processes which are produced by additional centripetal stimulation. These changes proved to be complex and to vary significantly with the duration and frequency of the additional stimulation. Their interpretation is beset with the fundamental difficulty that one cannot with certainty state whether a decreased activity represents an increase of the inhibitory process or a decrease of the excitatory process; or, vice versa, whether an increased activity represents a decrease of the inhibitory process or an increase of the excitatory process. This difficulty is increased by the fact that the excitatory and inhibitory effects of the additional centripetal stimulation may be expected to exhibit on the one hand summation (recruitment) and on the other hand fatigue, both of which will vary with the frequency and duration of the stimulation.

*Impairment of inhibition.* The impairment of inhibition described above (p. 322) was reversible and should be attributed to the central nervous sys-



tem since the conditions of the experiments did not tax conduction in nerve axons. Fatigue is defined as a reversible impairment of performance due to previous activity. A reversible central impairment of the inhibition produced by stimulation of a nerve trunk could result from fatigue of the inhibitory process, from excitatory recruitment, or from fatigue of the excitatory process at some interneuronal synapse involved in the inhibitory reflex (2). I have avoided using the term "fatigue of inhibition" because it may imply a fatigue of the inhibitory process to the exclusion of these other possibilities.

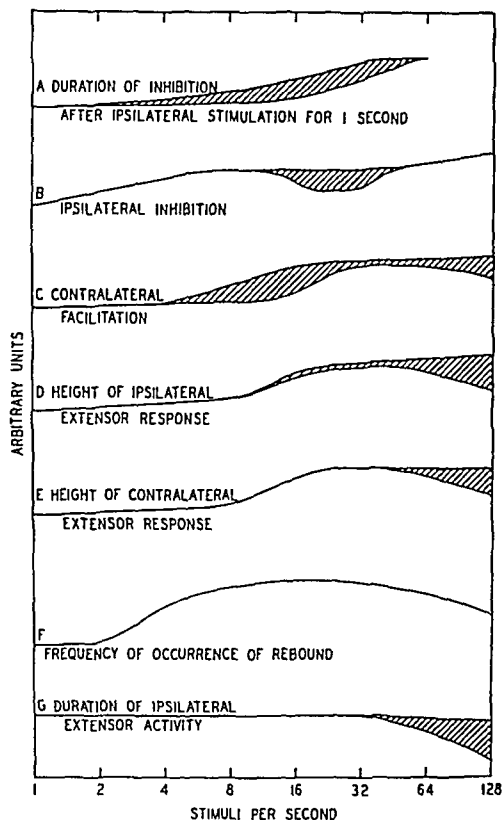


FIG. 9. A diagrammatic representation of the relation of the reflex effects upon the quadriceps muscle to the frequency of centripetal stimulation of sensory nerves in the hind limbs. With the exception of F, each of these curves is based upon experiments in which the reflex effect labeled on the curve was observed at a number of frequencies of stimulation in the *same* preparation. Each curve, however, is typical of the preparations studied. The shaded areas represent the range of variability of response in different preparations. The ordinates of curves B and C represent effects which are measured throughout a period of centripetal stimulation for several minutes. The ordinates of curves D and E represent effects which occurred at the commencement of a period of centripetal stimulation. The ordinates of curve F represent the per cent of the preparations which showed excitatory rebound after centripetal stimulation of an ipsilateral nerve for one to five minutes. Compare curve A with Fig. 3; curve B with Figs. 1, 2; curve C with Fig. 6. Curve F is based on Fig. 8 and Table I.

If fatigue of the inhibitory process does play a predominant role, its time-course is slower than that of the excitatory process. For example, the rapid decline of the ipsilateral extensor responses often seen at frequencies of 60/sec. or more (Figs. 1, 2, curve G in Fig. 9) may be attributed to a rapid fatigue of the excitatory process; but the inhibition of the knee-jerks at these frequencies diminished only after a longer time (Fig. 4), suggesting a slow fatigue of the inhibitory process. An alternative explanation is that the impairment of the extensor responses results from an increasing intensity of the inhibitory process, *i.e.*, inhibitory recruitment (5). The impairment of inhibition in some preparations during the last two minutes of a four-

minute period of ipsilateral stimulation at about 8/sec. (Fig. 1) may indicate that excitatory recruitment has occurred, since a more rapid fatigue of the inhibitory process at low than at high frequencies is unlikely.

Tapping at a fast rate (3/sec.) does not usually cause a gradual increase in the size of the uninhibited knee-jerk. During ipsilateral stimulation, however, the impairment of inhibition was more rapid when the knee-jerks were elicited at this fast rate than at a slow rate (Fig. 5). At the end of stimulation, excitatory rebound occurred only if the tapping was fast. One may argue that the ipsilateral stimulation prolongs the excitatory process produced by each tap (beyond 1/3 second) by setting up activity in interneuronal circuits. Both the impairment of inhibition and the excitatory rebound would then result from excitatory recruitment. On the other hand, if the inhibitory and excitatory processes inactivate each other (6), it is unnecessary to invoke excitatory recruitment, and the impairment of inhibition would indicate a waning of the inhibitory process itself.

*Relations between stimulus frequency and reflex effects.* Figure 9 is a diagrammatic representation of the relation of the reflex effects upon the quadriceps muscle to the frequency of centripetal stimulation of sensory nerves in the hind limbs. An analytical comparison of the curves in this figure may enable one to estimate the relations of the intensities of the inhibitory and excitatory processes to stimulus frequency. Curves C, D, and E represent different excitatory effects. The similarity of these curves suggests that the principal factor determining their shape is the relation of the intensity of the excitatory process to stimulus frequency. One can generalize from these curves that the intensity of the excitatory process increases with frequency up to 30/sec. The rate of increase is slow at frequencies lower than 8/sec. At frequencies higher than 30/sec., the intensity of the excitatory process is probably about the same as that at 30/sec., but fatigue of the excitatory process complicates the interpretation at high frequencies.

The intensity of the inhibitory process probably increases continuously with frequency from 1/sec. to 120/sec. Thus at frequencies lower than 8/sec. the steady increase of ipsilateral inhibition with stimulus frequency (curve B, Fig. 9; see also Fig. 2) must result from an increasing intensity of the inhibitory process, since, as noted above, the intensity of the excitatory process is increasing rather than decreasing with stimulus frequency. At frequencies between 8/sec. and 30/sec. it may be concluded that the intensity of the inhibitory process increases with frequency since the duration of the inhibition following brief stimulation (curve A) was always greater at 30/sec. than at 8/sec. The decrease in degree of inhibition at 30/sec. with respect to that at 8/sec. (see shaded area in curve B) may have been caused by the greater intensity at 30/sec. than at 8/sec. of the excitatory process resulting from the ipsilateral stimulation. At frequencies higher than 30/sec. the increasing duration of inhibition following brief stimulation (curve A), and the increasing degree of inhibition (curve B) are primarily due to an increase of the intensity of the inhibitory process with stimulus frequency, since the

intensity of the excitatory process did not decrease sufficiently at high frequencies to explain the marked augmentation of both these inhibitory effects (compare curves C, D, and E with A and B).

### SUMMARY

An analysis has been made of the relations between the frequency of centripetal stimulation of a nerve-trunk and the resulting reflex effects upon the anterior horn cells innervating the quadriceps muscle in the acute spinal cat. These effects can be classified as: (i) ipsilateral extensor activity (p. 319), (ii) ipsilateral inhibition of the knee-jerk (p. 321), (iii) contralateral extensor activity (p. 323), (iv) contralateral facilitation of the knee-jerk (p. 322), (v) inhibitory after-effects (p. 321), and (vi) excitatory rebound (p. 323).

The intensity of the excitatory process increases with frequency from 1/sec. up to 30/sec., without further increase up to 120/sec. The rate of increase is slow at frequencies lower than 8/sec. The intensity of the inhibitory process increases with frequency from 1/sec. up to at least 120/sec., the highest frequency studied.

### ACKNOWLEDGMENTS

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# MECHANISM OF PUPILLARY DILATATION ELICITED BY CORTICAL STIMULATION

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## INTRODUCTION

SINCE Bochefontaine (1) first recorded the fact that pupillary dilatation may be elicited by electrical stimulation of the cerebral cortex, a vast literature has arisen concerning the role of sympathetic and parasympathetic pathways responsible for this phenomenon. Parsons (10) showed that the pupillary dilatation obtained by electrical stimulation of the arcuate gyrus in the monkey is diminished but not abolished by section of both cervical sympathetic chains. Moreover, Ury and Oldberg (18) suggest that dilatation of the pupil is brought about solely by inhibition of the Edinger-Westphal nucleus since they found that the inhibitory pupillary reaction obtained by stimulation of area 8 in the cat is unaffected by cervical sympathectomy. This same conclusion was also reached by Ury and Gellhorn (17) who postulate that, under normal conditions, the pupillary dilatation in response to pain is almost exclusively due to parasympathetic inhibition rather than to sympathetic stimulation. Because of the disagreement in the literature as to the mechanism concerned, further experiments have been performed on the monkey.

## METHODS

Experiments were performed on monkeys (*Macaca mulatta*) under Dial anesthesia, except in one instance when light ether anesthesia was employed. Electrical stimulation of the lateral and medial surfaces of the frontal lobe was carried out, using bipolar Ag-AgCl electrodes and the Goodwin stimulator. For the most part, stimuli at 40/second with a falling phase of 2 sigma were employed.

In one animal, bilateral cervical sympathectomy was performed under aseptic conditions, the superior cervical ganglion and 3 cm. of sympathetic chain being excised bilaterally, the cerebral cortex being stimulated at a later date and pupillary responses recorded. In other animals unilateral cervical sympathectomy of the same extent and section of the third nerve by the subtemporal approach were carried out acutely. Pathways within the central nervous system were investigated by the method of strychninization, the intracerebral, bipolar electrode being accurately placed by means of the Horsley-Clarke stereotaxic instrument and the potentials of the deep structures and cortex—following local application of strychnine to a small area of cortex—being recorded on a 6-channel, ink-writing Grass oscillograph. Stimulation of deep structures was carried out, using the same type of electrode and the Goodwin stimulator.

## RESULTS

*Cortical stimulation.* Electrical stimulation of the frontal cortex around the anterior tip of the superior limb of the arcuate sulcus yields consistent bilateral pupillary dilatation, usually without ocular movement. Occasionally slight elevation of the upper lids ("awakening reaction") with a definite

blink at the end of stimulation is obtained in addition. These responses can also be elicited from the region extending medially from the tip of the arcuate sulcus over onto the medial surface of the superior frontal convolution and throughout the extent of Brodmann's area 32, occasional responses being obtained just below the cingulate sulcus in area 24. Points slightly more posterior on the arcuate gyrus along the superior limb yield bilateral pupillary dilatation and conjugate deviation of the eyes, usually to the opposite side. Under Dial anesthesia, the resting pupil measures about 3 mm. Upon cortical stimulation, after a latency of 3-4 seconds, there is rapid and marked pupillary dilatation to about 8 mm. with a quick contraction to the resting

diameter of the pupils under bright illumination is about 6 mm. Following a short latency of 1-2 seconds, cortical stimulation yields a prompt dilatation to 8 mm. These responses have been described many times by Smith (15) and other workers.

*Sympathectomy.* Unilateral cervical sympathectomy uniformly abolished the pupillar dilatation in the sympathectomized eye upon cortical stimulation of either hemisphere, a maximal dilatation in the normal eye still being elicitable. One week following bilateral cervical sympathectomy, extensive cortical stimulation under Dial anesthesia failed to elicit any pupillary dilatation whatever, even with strong stimuli, although ocular movements were elicited with ease. Following unilateral cervical sympathectomy under light ether



FIG. 1. Location of intracerebral electrode along lateral surface of oculomotor nucleus. Weil stain.

anesthesia, a minimal pupillar dilatation (0.5-1.0 mm.) could be obtained in the sympathectomized eye on cortical stimulation, as compared with a dilatation of 4 mm. in the normal eye. It would thus seem possible that the active component of pupillary dilatation elicited by cortical stimulation travels over the sympathetic system, a minimal inhibition of parasympathetic tone also being produced of the same order as that which obtains in reciprocal innervation elsewhere in the nervous system.

*Section of the oculomotor nerve.* Section of the third nerve intracranially under Dial anesthesia causes a typical ophthalmoplegia with dilatation of that pupil to 7 mm. Subsequent cortical stimulation, anywhere in the area delimited above, continues to cause prompt dilatation of both pupils, the

parasympathectomized pupil now dilating from 7 to 10 mm. while the normal pupil continues to dilate from 3 to 9 mm. Subsequent unilateral cervical sympathectomy on the same side abolishes all pupillary dilatation in the previously parasympathectomized pupil. It is thus demonstrated that the third nerve is not a necessary component of the pupillo-dilator response elicited by cortical stimulation.

*Pathways.* Since minimal inhibition of constrictor tone in a sympathectomized pupil can be obtained by cortical stimulation under special conditions of anesthesia, the pathway by which this effect is mediated was studied by the strychnine method.

A bipolar recording electrode was placed in the oculomotor nucleus (Fig. 1) and local strychninization of several square millimeters of cortex in the region of the anterior tip of the arcuate sulcus carried out. Local strychninization of the cortex just medial to the tip of the arcuate sulcus, from which maximal pupillary changes are elicitable by electrical stimulation, resulted in typical firing in the oculomotor nucleus (Fig. 2). Nearby strychninizations elsewhere in this region were without effect. The area from which firing was obtained corresponds very closely with area 8 while the adjacent strychninizations fall in area 9. Thus a direct projection from area 8 to the oculomotor nucleus is demonstrated. It

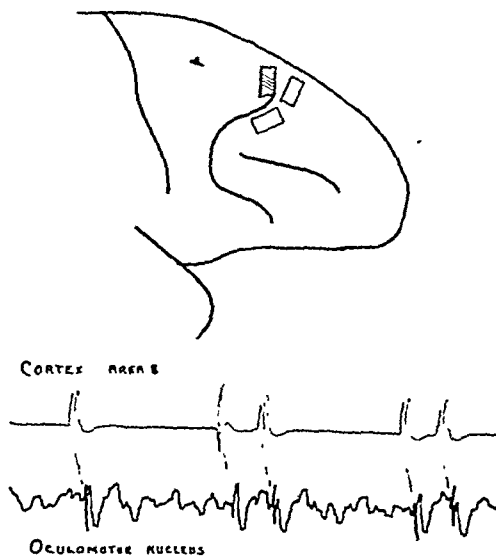


FIG. 2. Electrical record obtained on strychninization of area 8. Shaded area is the only one which fires oculomotor nucleus.

would seem probable that the minimal inhibition of activity in the oculomotor nucleus obtained by cortical stimulation is transmitted over this pathway.

A limited investigation has also been carried out to delimit the pathways involved in the active pupillary dilatation mediated over the peripheral sympathetic nervous system. A bipolar electrode was placed in the lateral hypothalamic area, stimulation in this region having been shown by Hodes and Magoun (4) to produce bilateral pupillary dilatation. As noted above, cortical stimulation of an animal lightly anesthetized with ether in which the cervical sympathetic chain has been removed unilaterally results in maximal dilatation in the normal pupil with minimal dilatation of the sympathectomized pupil. Hypothalamic stimulation in the same preparation caused prompt maximal dilatation of the normal pupil alone. This would tend to indicate that the lateral hypothalamus in a sense approximates the final

common path as regards the active component of pupillary dilatation and is not concerned with the reciprocal inhibition of the oculomotor nucleus which has been demonstrated from area 8 of the cortex. Multiple strychninizations of the frontal cortex, including area 8, failed to fire into this region, indicating that if the lateral hypothalamus is involved in the pathway between cortex and sympathetic chain, there is more than one synapse between cortex and it. A rather exhaustive investigation of all levels between cortex and bulb would be necessary to define the details of this multi-neuronal pathway.

### DISCUSSION

Much of the previous controversy regarding the mechanism of pupillary dilatation has centered on the reflex dilatation of the pupil elicited by stimulation of peripheral nerves. This particular aspect has been reviewed by Ury and Gellhorn (17) who conclude that under normal conditions the pupillary dilatation in response to pain is almost exclusively due to parasympathetic inhibition but that, under conditions which increase the excitability of the cilio-spinal center, sympathetic discharges may contribute materially to the dilatation. Kuntz and Richins (7) postulate that the pupillodilator response elicited by peripheral nerve stimulation is mediated through the mesencephalic parasympathetic center where it is actively integrated and controlled. They further suggest that the inhibition of the circular muscle of the iris is brought about, not by inhibition of the oculomotor nucleus, but by activation of this center which results in the discharge of efferent nerve impulses through adrenergic fibers arising in the ciliary ganglion. The afferent pathways for this reflex have been further defined by Harris, Hodes and Magoun (3) who demonstrated by localized lesions that the afferent impulses pass up the cord in the lateral spinothalamic tract, leaving it in the bulb to pass dorsally through the bulbar reticular formation to the dorsal tegmentum, where they conclude that the afferent fibers end in the region of the oculomotor nucleus since sections of the brain rostral to the oculomotor nucleus were without effect on the pupillodilator reflex. Ury and Oldberg (18), however, felt that this reflex could be modified by large post-central lesions in the cortex.

In the present investigation it has been demonstrated that the pupillary dilatation elicited by electrical stimulation of area 8 of the monkey's cortex is mediated by the sympathetic nervous system, minimal dilatation due to inhibition of the oculomotor nucleus being obtainable only under special conditions of anesthesia. The relationship between these two sets of observations is not immediately apparent. The pupillary dilatation obtained by inhibition of the oculomotor nucleus from cortical stimulation is 0.5–1.0 mm. which is of the same order as the reflex dilatation obtained by Ury and Gellhorn from sciatic nerve stimulation, while the cortically induced dilatation which travels over the sympathetic nervous system is of the order of 5–6 mm. It may thus be possible that minimal or small adjustments in pupillary diameter are mediated by changes in the constrictor tonus of the oculomotor

nucleus, while the large, maximal responses obtainable from the cortex are purely sympathetic in their mediation.

Since this cortical response involves reciprocal innervation, the relative tone of lower sympathetic and parasympathetic structures will, to a certain extent, determine the mechanism of the response as also is the case with antagonistic responses in the somatic field. Recent studies by Siebens and Woolsey (14) in the cat confirm the findings of the present investigation and thereby rule out species difference as an essential factor.

François-Franck (2) noted that cortically induced pupillary dilatation is abolished by cervical sympathectomy, although he did admit a slight degree of dilatation in the sympathectomized pupil which he ascribed to dilator fibers running another course, probably in the trigeminal nerve. Parsons (10) was unable completely to confirm this in either the dog, cat or monkey. Although cortically elicited retraction of the nictitating membrane, widening of the palpebral fissure and projection of the eyeball he found to be abolished by previous section of the cervical sympathetics, pupillary dilatation was only moderately diminished. He concluded that, in the sympathectomized pupil, the effect is probably due to an inhibition of the tonic action of the third nerve "in the absence of the usual dilator tracts." Ury and Oldberg (18) stated that a dilatation of 2 mm. could still be elicited by electrical stimulation of area 8 in the cat following unilateral cervical sympathectomy.

On the basis of the present investigation, it seems logical to assume that the pupillary dilatation obtained from cortical stimulation is achieved by the mechanism of reciprocal innervation so well defined by Sherrington, the active component of dilatation being a localized cortical sympathetic response, the antagonistic constrictor component of the oculomotor nucleus being concomitantly inhibited. This type of phenomenon has been shown to hold true for extraocular movements by Sherrington (13); he also assumed that it would hold true for the pupillary dilatation elicited from the cortex and was somewhat disappointed to find that such dilatation was completely abolished by cervical sympathectomy in the two monkeys in which he attempted the experiment, no evidence of oculomotor inhibition being evident under his experimental conditions.

The inhibitory component of the cortical response has been demonstrated to travel by a single neuron linkage from area 8 to the oculomotor nucleus. Fibers from the general vicinity of area 8 were found to pass to the eye-motor nuclei by Mettler (9), while Levin (8) demonstrated degeneration from area 8 descending into the cerebral peduncle, passing through the region of the substantia nigra and into the tegmentum, but he could not trace them into the superior colliculi. By stimulation methods, Hodes and Magoun (4) showed that inhibition of the oculomotor nuclei could also be obtained from a wide area in the hypothalamus, the basal telencephalon, the midline part of the thalamus, the subthalamus and a large part of the midbrain.

The intimate details of the active dilator component of the cortical response are as yet incompletely understood. Electrical stimulation in the



lateral hypothalamus yields pupillary dilatation without inhibition of the oculomotor nucleus. Ingram, Ranson and Hannett (5) demonstrated the areas of the hypothalamus from which pupillary dilatation can be elicited, and this has been further defined by Hodes and Magoun (4) who demonstrated that the points for sympathetic pupillary dilatation were, for the most part, interspersed with those points from which parasympathetic inhibition could be obtained. It has been demonstrated here that area 8 does not project directly into this region of the hypothalamus and this is confirmed by the anatomical studies mentioned above. That this region is a necessary component of the pupillo-dilator pathway has been indicated by Karplus and Kreidl (6) who found that the dilator response from the cortex in cats is abolished by destruction of the hypothalamus.

Pupillary dilatation elicited by cortical stimulation is thus another example of a localized cortical sympathetic response. Similar responses have been noted by Schwartz (12) who demonstrated that the fall in skin-resistance elicited by various external stimuli in the cat is abolished by excision of area 6, and by Smith (16) who demonstrated localized pilomotor responses from area 24. That localized sympathetic responses are present near the conscious level in man has been shown by Petrovic and Tschernomolossow (11) who report one case in which the act of recalling a painful or fearful experience was associated with so-called "voluntary" pupillary dilatation.

### CONCLUSION

Pupillary dilatation elicited by electrical stimulation of area 8 in the cerebral cortex of the monkey is a localized sympathetic response having all the properties of reciprocal innervation. This response is abolished by section of the cervical sympathetic chain, minimal pupillary dilatation due to inhibition of the oculomotor nucleus being obtainable only under special conditions of anesthesia. The active dilator component travels over pathways involving the hypothalamus. This cortico-hypothalamic pathway is not a direct one. The reciprocal inhibitory pathway from area 8 to the oculomotor nucleus has been shown to be a direct projection.

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# INHIBITION AT THE NERVE MUSCLE JUNCTION IN CRUSTACEA

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IN A PREVIOUS PAPER the response of crustacean muscle to motor nerve impulses was studied (7). It was shown that the junctional potentials were similar to those observed in vertebrate muscle. Depending upon the number and frequency of nerve impulses, however, local as well as propagated muscle responses are set up. In the present paper, effects of inhibitory nerve impulses on the junctional potential changes and on the subsequent muscle responses are examined. Marmont and Wiersma (9), in a very interesting study, reported that by different timing of the inhibitory impulse, relative to the excitatory, complete mechanical inhibition can be obtained, with or without reduction of the electric response. In the light of our recent findings it seemed appropriate to re-examine this effect. In view of the general similarity of synaptic potentials in vertebrates (3) as well as crustacea, it is hoped that this investigation will have some bearing on the problem of central inhibition in higher animals.

## METHODS

The general technique has been described previously (7). Three muscles were used for the present work: the opener of the claw and the extensor and flexor of the dactylopodite (crab and crayfish). The opener and extensor have the advantage of a more complete mechanical inhibition, but the disadvantage of a less convenient nerve supply than the flexor (Fig. 1).

It was essential for most experiments to stimulate motor and inhibitor axons separately. After isolation from the meropodite, the limb nerve separates readily into two main branches, a thick and thin bundle (Fig. 1). At this stage, a number of difficulties arise: (i) In the crab, motor and inhibitor axons to the opener and extensor are adjacent and usually difficult to separate. In some preparations, when separation did not seem feasible, two pairs of electrodes were placed at different distances on the nerve bundle, and use was made of a threshold difference of the two fibres to stimulate them separately. This procedure, however, is not very satisfactory and only permits of a limited variation of intervals between the two impulses. The presence and possible stimulation of more than one inhibitor (11) does not affect the general results or arguments. (ii) In the crayfish (Fig. 1), motor and inhibitor fibres to the opener run in separate bundles, but the inhibitor is adjacent to the motor axon(s) supplying the closer. In some experiments, the closer muscle was removed, but this operation usually resulted in damage to the opener and its nerve supply. Separation of the nerve axons during their common course succeeded occasionally, but was rather difficult because of fairly strong connective tissue. It was simplest to crush the nerves to the closer near their entry into the muscle. (iii) The flexor preparation was the easiest to obtain but the mechanical inhibition was usually incomplete, a remainder of about 20 to 30 per cent of the tension being left, when motor and inhibitor nerves were stimulated at the same frequency. To eliminate responses of the extensor muscle, the latter was either removed or its nerve supply cut.

Strip preparations containing a small number of muscle fibres, as obtained in the ex-

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tensor of the carpopodite (7), were difficult to prepare and were only rarely used. In most experiments, recording was done on the intact opener of the claw. On the outer surface, many muscle fibres could be exposed over several mm. length without injury. Foci of nerve terminations could then be found by small movements of the "active" electrode. Regular

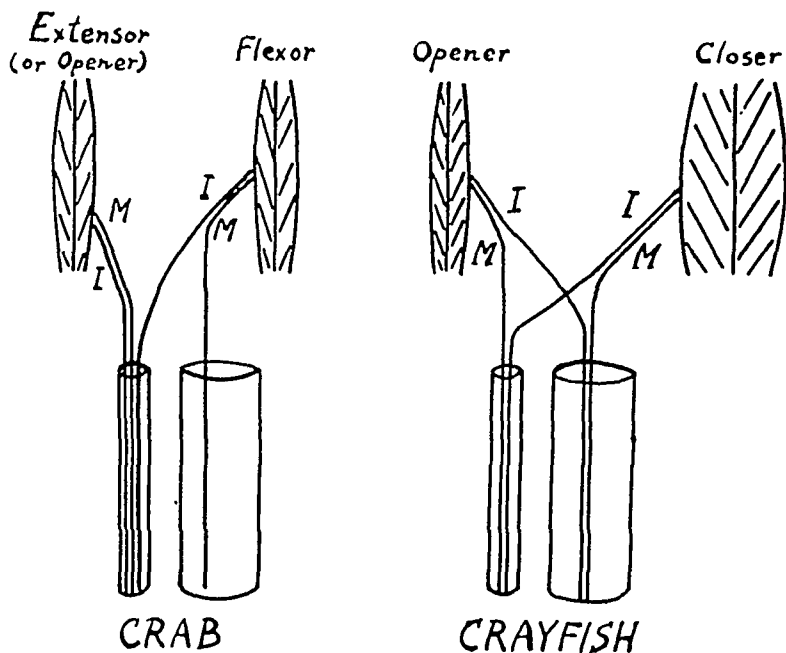


FIG. 1. Diagram illustrating course of motor (*M*) and inhibitory (*I*) axons in crab and crayfish.

checks were made against extraneous action potentials, coming from the extensor and flexor of the propodite. Occasionally it was necessary to cut the tendons of those muscles and allow them to retract.

## RESULTS

### *A. Response to stimulation of inhibitory axon only*

If the inhibitory axon is stimulated alone, the only response which can be seen, even with very high amplification, is a small intramuscular nerve spike, similar to the motor nerve spike preceding the endplate potential (e.p.p.) This little spike is a useful indication (i) of the effectiveness of stimulation and (ii) of the time of arrival of the inhibitory impulse at the junction relative to the motor impulse (section B). The absence of any detectable response of the resting muscle to inhibitory stimulation agrees well with previous observations (9). (See Figs. 2 and 3.)

If the recording electrodes are placed on a small nerve bundle which contains the inhibitory axon, a fairly large nerve spike is seen which does not differ in any important respect from the motor nerve spike.

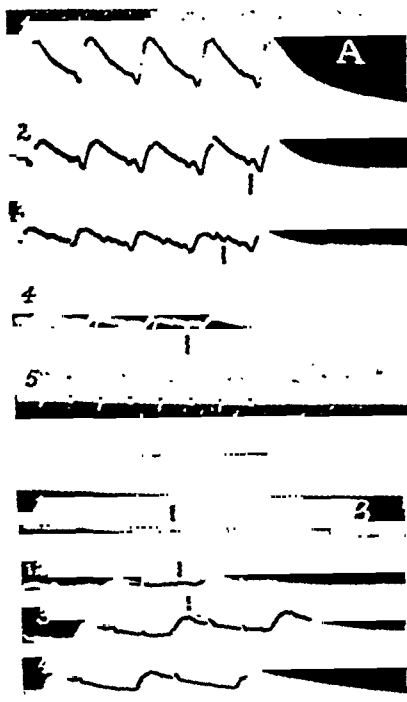
A consistent difference was observed in the response of motor and inhibitory axons to mechanical stimulation. During attempts to separate motor and inhibitory nerves to the

opener of the claw and to the extensor of the dactylus in the crab, accidental mechanical stimulation of the nerve frequently caused strong contractions. Recording from the muscles showed that the contractions were due to repetitive nerve discharges, setting up local and propagated responses. Electrical stimulation of the two nerves at 50 per sec. abolished the propagated spikes and the contraction, but e.p.p.'s were still recorded (see later). Simultaneous crushing of motor and inhibitory nerve fibres usually gave an uninhibited contraction. It therefore seems that the inhibitory axon is much less excitable by mechanical stimuli than the motor.

### B. Effect of inhibitory impulse on motor response

The ideal experimental set-up would be to apply a *single* motor impulse at various intervals before and after a *single* inhibitory impulse and to study

FIG. 2.  $\alpha$ -action: Stimulation at 50 per sec. Opener of crayfish claw. Leading from exposed muscle. A, 15°C. 1, motor stimulation. Note: decay of potential indicates a slow component, probably due to contraction and slight movement of the electrode. Small positive deflexion at beginning of e.p.p. indicates that lead was slightly off focus. 2-4, motor and inhibitory stimulation at different intervals. Position of last inhibitory nerve spike is indicated by dashes. Maximum  $\alpha$ -action at 4. 5, inhibitory stimulation only, showing nerve spikes. B, Two different M-I intervals. Marked  $\alpha$ -action in 2, little or no  $\alpha$ -action in 3. 4, motor stimulation only. Inhibitory nerve spikes shown by dashes.



their interaction. In practice, it is necessary to use repetitive stimuli, because in most muscles one or a few motor impulses do not suffice to build up a large enough electrical and mechanical response. The only exception is the "twitch-system" of the claw, but on this the inhibitor has little or no effect. If both motor and inhibitory nerve impulses are set up at, say, 50 per sec., two separable actions are observed.

(i) *Electrical inhibition.* The electric response of the muscle is reduced to a variable extent, depending upon the relative time of arrival of inhibitory (*I*) and motor (*M*) impulses at the junction. The effect is greatest when *I* precedes *M* by about 2 msec., but is almost nil when *I* arrives after *M*. This electrical inhibition will henceforth be called  $\alpha$ -action, or, for reasons given later, "curare-like" action of the inhibitor. By reducing the e.p.p., the  $\alpha$ -action has two indirect effects, (a) it prevents the setting up of propagated muscle impulses, (b) it diminishes or abolishes local contraction.

(ii) *Direct mechanical inhibition.* Even if *I* arrives after *M* too late to affect the e.p.p., it is still capable of abolishing the local contraction in the vicinity of the nerve endings, by means of a direct action which was first described by Marmont and Wiersma (9). This action will henceforth be called  $\beta$ -action.

1. "CURARE-LIKE" ACTION ( $\alpha$ -ACTION) OF INHIBITORY IMPULSE. If the inhibitory nerve impulse arrives at the muscle slightly before the excitatory, the size of the e.p.p. is reduced appreciably. During steady stimulation at 50 per sec. the maximum reduction observed in the opener of the claw or extensor of the dactylus was about 80 per cent (see Fig. 2A<sub>1</sub>).

*Time course of  $\alpha$ -action.* As the interval between the two nerve impulses [inhibitory (*I*) and motor (*M*)] is increased, the  $\alpha$ -effect gradually diminishes, as shown in Figures 2 and 4. This agrees well with the findings of Marmont and Wiersma (9). At 17°C., the inhibitory action declines within 20 to 25



FIG. 3. Same experiment as Fig. 2A. Time scale 20 msec., voltage scale 2 mV. 1, inhibitory stimulation only. 2, three inhibitory impulses. 3, one inhibitory impulse interposed during the series. 4, motor stimulation only. Inhibitory impulses indicated by dashes. Note: occasionally small muscle spikes are present, indicated by arrows.

milliseconds ( $\frac{1}{2}$  decay in about 5 msec.). The time course of this effect happens to be much the same as that of the e.p.p., though this can hardly be more than chance.

While the size of the e.p.p. is reduced by the  $\alpha$ -action, depending upon the *M-I* interval, the time course of the e.p.p. is not appreciably affected. In particular, there was no evidence for any consistent change in the time course of decay of the e.p.p. at any *M-I* interval. In some experiments (e.g., Fig. 2A<sub>1</sub>), a slow component of potential was seen during motor stimulation, which was absent in the inhibited muscle. This, however, was not a consistent phenomenon; it depended greatly upon the strength of contraction and may well have been due to a small passive movement of the recording electrode.

In many experiments, at 50 per sec., little or no  $\alpha$ -effect is left behind by the preceding inhibitory impulses, as can be seen from Figures 2B and 4 (cf. also 9). In other cases, the time course of the  $\alpha$ -action is somewhat slower, and a slight degree of summation occurs. This shows itself in several forms.

(i) With steady stimulation, there is a residual small e.p.p. reduction (for example, 10 per cent), even if *I* follows *M* after a few milliseconds. (ii) If a few inhibitory impulses at 50 per sec. are interposed during a motor series, two impulses are required to give the full  $\alpha$ -action. The first impulse, if suit-

ably timed, produces a large reduction of the immediately following e.p.p. but this reduction becomes slightly greater with the next inhibitory impulse (Fig. 3<sub>2</sub>). (iii) Similarly, after cessation of inhibitory stimulation, the first e.p.p. reaches about 90 per cent while the second e.p.p. increases to the full normal size (Fig. 3<sub>3</sub>).

It is interesting to note that, at the end of an initial period of inhibitory stimulation, the e.p.p. almost immediately reaches the amplitude to which it would have grown in the absence of inhibition (cf. 9). It is clear, therefore,

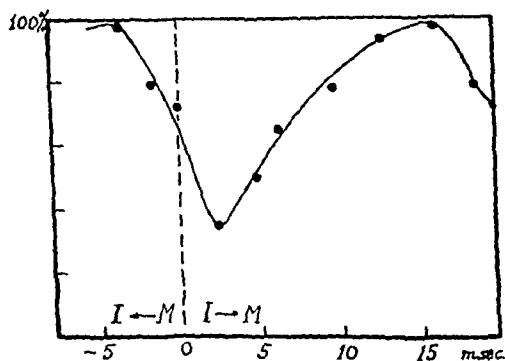


FIG. 4. Time course of  $\alpha$ -action. Temp. 17°C. Ordinates: Relative size of e.p.p. (uninhibited e.p.p. taken as 100%). Abscissae: Interval between inhibitory (*I*) and motor (*M*) impulse (using intra-muscular nerve spikes as indication). Positive values, if *I* precedes *M*. Frequency of stimulation was about 50 per sec.; hence the curve repeats at intervals of about 20 msec.

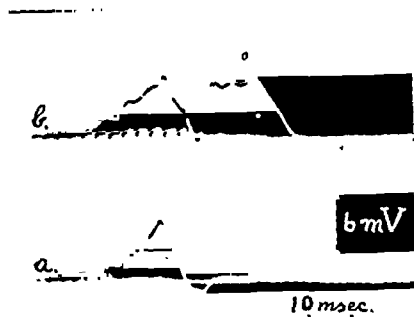


FIG. 5.  $\alpha$ -action at high frequency: Crab; opener of claw. 18°C. Whole muscle, recording from a junctional focus. *a*: Stimulation at 320 per sec. (nerve responds  $\frac{1}{2}$  to every second shock). Normal and inhibited responses are superimposed. *b*: same as *a*, except longer series of stimuli. Note: reduction of e.p.p. to a few per cent, hence spike abolished.

that the facilitation process which underlies the initial growth of the e.p.p.'s (see below) is not affected by the inhibitor.

A corollary is seen in Figure 6A, showing that at low frequencies the re-development of tension at the end of inhibition is somewhat quicker than the initial development. The higher speed is observed only during the "foot" of the tension curve, the later portions still rising in a slow and protracted manner. It seems reasonable to attribute the difference to the fact that, at the end of inhibition, the e.p.p.'s regain their full size almost at once, while initially they require about  $\frac{1}{2}$  sec. or more to grow up (7).

*$\alpha$ -effect at high frequencies.* At frequencies above 100 per sec., the  $\alpha$ -effect is greatly intensified by summation. Motor nerve stimulation in the opener of the claw and extensor of the dactylus at 160 per sec. builds up junctional potentials which normally give rise to propagated muscle impulses. Simultaneous stimulation of the inhibitory nerve reduces the e.p.p.'s to several per cent. As a consequence of the e.p.p. reduction, propagated spikes are completely abolished (Fig. 5).

Similar results were obtained from the flexor of the dactylus. In this mus-



cle, the reduction of the e.p.p. at high frequency was not as striking as in the extensor, or opener, but it was still sufficient to cut the spike activity down to a small fraction. It has previously been reported that inhibition in the flexor of the dactylus becomes less marked at high frequency (10), in contrast with the extensor or opener of the claw.

2. INACTIVATION OF CONTRACTILE MECHANISM BY INHIBITORY IMPULSE ( $\beta$ -ACTION). If the nerve fibres to the extensor of the dactylus, or opener of the claw, are stimulated at 50 per sec., complete mechanical inhibition is usually obtained, *independent* of the *M-I* interval. This confirms the observation by Marmont and Wiersma (9), *viz.*, that the inhibitory nerve impulse can

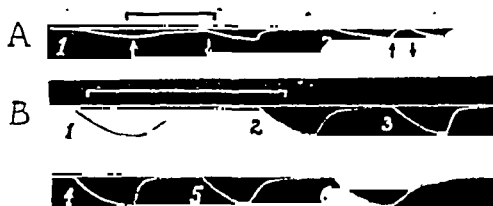


FIG. 6. Tension records of opener of crayfish claw. 17°C. Time scale 20 sec. A, stimulation at 10 per sec. After initial contraction inhibition applied and withdrawn, as indicated by arrows. *I-M* intervals: 1, 50 msec.; 2, 0 msec. Note: in 2, inhibition is as fast as relaxation; in 1, inhibition is much slower. B, stimulation at 20 per sec. After initial contraction inhibition is applied, and when complete, *I* and *M* are withdrawn simultaneously. *I-M* intervals: 1, 25 msec.; 2, -1.7 msec. (*M* preceding); 3, 2.5 msec.; 4, 0 msec.; 5, 5 msec.; 6, 12.5 msec.

abolish the contraction without noticeable alteration of the "action potential" of the muscle. (This statement is correct with one qualification: it is applicable to e.p.p.'s and local contractions only.) Thus, there are two separate actions of the inhibitor: one interfering with the production of the e.p.p. and another acting directly on the contractile process, at the junctional region. It should be emphasized that complete mechanical inhibition is observed only in the absence of muscle spikes. Once a propagated spike has been set up and has begun to travel away from the junction the inhibitory impulse cannot prevent it from eliciting a twitch.

An observation which is of interest in this respect was made on the extensor of the carpopodite. When stimulating motor and inhibitory axons at 40 per sec., the e.p.p.'s were not always sufficiently reduced to prevent the appearance of occasional spikes. Mechanically, this was accompanied by occasional fast twitches, while the maintained local contraction which accompanies the e.p.p.'s in uninhibited muscle was absent.

*Rate of relaxation and of mechanical inhibition.* At 50 per sec. and higher frequencies, the mechanical effect of inhibition was usually indistinguishable from normal relaxation at cessation of the motor stimuli, and its rate was (cf. 10) independent of the *M-I* interval. Occasionally mechanical inhibition appeared to be even more rapid than relaxation. In these cases, however, "relaxation" was accompanied, and slowed, by after-discharges in the muscle. The exact origin of these after-discharges is not quite clear, but they could be stopped by simultaneous stimulation of the inhibitory axon. To obtain some idea of the time course of the  $\beta$ -effect, very low frequencies (10-

20 per sec.) had to be used. It became then possible to increase the  $M$ - $I$  intervals sufficiently to observe a noticeable change in the mechanical inhibition. Although the size of the contractions at 10–20 per sec. is very small, the results were quite definite, as illustrated in Figure 6.

The opener of the crayfish claw was used in these experiments. The tendon of the closer was cut, and the dactylus connected to a light tension lever. The motor nerve was stimulated at 10 or 20 per sec., and after a few seconds

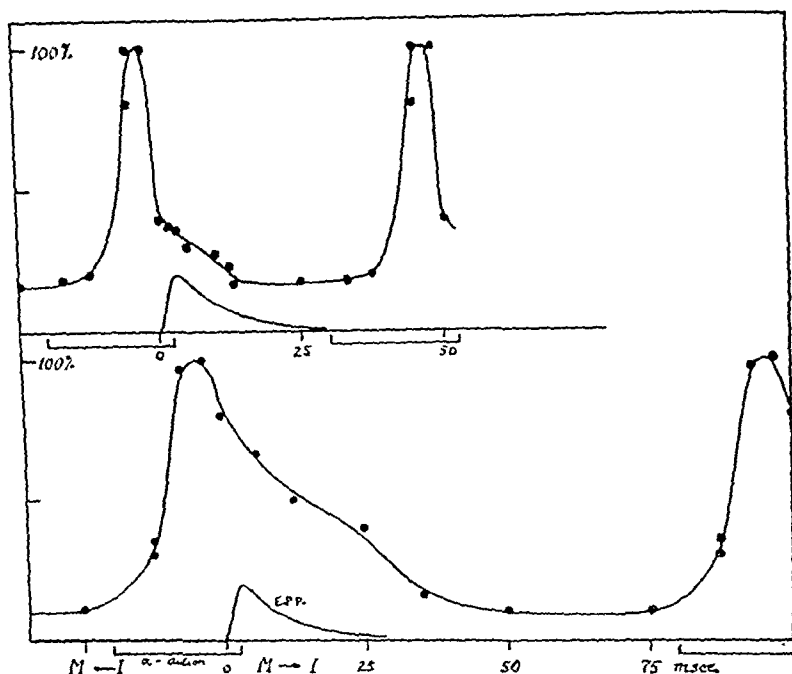


FIG. 7. Relation between rate of mechanical inhibition and  $M$ - $I$  interval. Ordinates: Rate of tension decline from 90 to 50 %. Normal relaxation rate taken as 100 %. Abscissae:  $M$ - $I$  intervals. Positive values if  $I$  follows  $M$ . Opener of crayfish claw. 17°C. Frequency of stimulation was 10 per sec. (lower figure) and 20 per sec. (upper figure); hence the curves repeat at 100 and 50 msec. respectively. For comparison approximate time course of e.p.p. and  $\alpha$ -action are shown.

inhibitory stimuli were added. When inhibition had reached a steady state, both stimuli were withdrawn or inhibition alone was stopped and the redevelopment of tension was observed (Fig. 6). To express the rate of relaxation and inhibition numerically, the time required for a certain percentage drop of tension (e.g., from 90 to 50 per cent) was measured, its reciprocal being an indication of the rate. The rate of inhibition varies with the  $M$ - $I$  interval in a characteristic manner. When  $I$  slightly precedes  $M$ , inhibition is as rapid as normal relaxation (Fig. 6A<sub>2</sub> and 6B<sub>3</sub>). When  $I$  follows or precedes  $M$  at increasing intervals, the rate of inhibition drops (Fig. 6A and 6B). If the rate is plotted against the  $M$ - $I$  interval (Fig. 7), a curve is obtained which shows two distinct components: (i) A quick phase, lasting about 25 msec.,

which reaches a sharp peak with *I* preceding *M* by 2-3 msec. This coincides with the period during which the e.p.p. is depressed. Obviously, the fast component is the mechanical counterpart of the  $\alpha$ -effect and indicates that the reduction of the e.p.p. is accompanied by reduced activation of the contractile substance. (ii) There is a prolonged phase which declines slowly *after* the fast  $\alpha$ -component has disappeared. It lasts longer than the e.p.p. and is able to sum, even at 10 per sec. This is the  $\beta$ -effect proper, and is presumably due to direct inactivation of the contractile substance by the inhibitor.

### DISCUSSION

Histological evidence shows that the motor and inhibitory nerves run closely together after entering the muscles. Their common course can be followed down to the fine intrasumcular branches. This was observed in our methylene-blue preparations and has also been noted by others (4). No accurate histological study has yet been made of the terminations of the different nerves. It seems likely, however, that motor and inhibitory fibres not only run closely together, but terminate in close proximity on the muscle. This is suggested also by the prompt inhibitory effect, which may take place within one or two milliseconds after the arrival of the inhibitory impulse (Figs. 2 and 3).

The question arises as to which links in the neuromuscular transmission chain are broken by the inhibitory impulse. It is evident that the  $\alpha$  and  $\beta$  actions interfere at different phases of transmission.

1. The  $\alpha$ -action is similar to the curarine effect in vertebrate muscle. It reduces the e.p.p. without apparently altering its time course, and it does not, by itself, change the resting potential of the muscle. It is not intended to suggest that the inhibitor might be a curare-like compound; on the contrary, it is well known that curarine does not block neuromuscular transmission in crustacea (6, 7). It is reasonable to assume, however, that the  $\alpha$ -inhibitor is an agent which interferes with the *production* of the e.p.p. It may, like curarine, prevent the depolarizing action of the transmitter at the endplate region. This process could be visualized as a competitive action between inhibitor and transmitter on the muscle membrane. Such an effect has been observed in frog's muscle where curarine can prevent or abolish the depolarization caused by drugs like nicotine or acetylcholine (2, 8).

It is significant that the  $\alpha$ -action vanishes if the inhibitory impulse arrives only slightly later than the motor, still during the early portion of the e.p.p. but presumably too late to affect the building up of the e.p.p. by the transmitter.

The inhibitor does not depress or delay the facilitation process which is responsible for the growth of successive e.p.p.'s (section B1, also 9). One may conclude therefore that the e.p.p. facilitation is due to some process very early in the transmission chain, definitely preceding the stages on which the inhibitor can act. It has been suggested previously (7) that the mechanism on which the initial growth of e.p.p.'s is based, may be prior even to the libera-

tion of the transmitter substance. As the inhibitor probably acts on the receptors of the muscle membrane (see above) it is not surprising that the *release* of the transmitter and any event prior to it remain unaffected.

2. The  $\beta$ -action is apparently due to the inhibitor interfering locally with the mechanical activation process. We are confronted with a puzzling problem, namely, the existence of an inhibitor which has no electric effect on the muscle membrane (no change of resting potential or time factor), and yet is capable of acting on the contractile substance itself. At the present state of our knowledge it is difficult to imagine how this action is brought about.

From Figure 7 one might be tempted to conclude that the  $\beta$ -action of the inhibitor is much slower than the  $\alpha$ -action. There is, however, no real evidence for this; the duration of the  $\beta$ -effect may well be determined by the time course of the inhibitory substrate, in this case presumably of the mechanical activation process following each e.p.p. This may be of the order of 0.1 to 0.2 sec. (1, 5, 7).

The apparent absence of the inhibitory effect on the "fast" system, for instance in the closer of the claw, is surprising, and one might suspect that the nerve terminations producing the "fast" contractions may not end in such close proximity with the inhibitor endings as the nerves responsible for the "slow" muscle response (7).

#### SUMMARY

The effect of inhibitory nerve impulses on the potential changes and on the subsequent contractions of crustacean muscle was investigated.

1. The action potentials of inhibitory axons do not differ from those of other nerve fibres. Inhibitory impulses alone have no detectable electrical effect on the muscle. These findings confirm those of other investigators.

2. There are two separate actions of inhibitory impulses on the motor response.

a. An electrical effect, the  $\alpha$ -action, reduces the junctional potential (e.p.p.) to an extent depending upon the relative times of arrival of inhibitory and motor impulses at the junction. Due to the reduction of the e.p.p. (i) the setting up of propagated muscle impulses is prevented and (ii) local contractions at the junctional region are diminished or abolished. At 17°C. that  $\alpha$ -action lasts about 20 to 25 msec. and declines to half in about 5 msec. The time course of the e.p.p. is not appreciably altered by inhibition.

b. The inhibitory impulse has direct action on the contractile process at the junction, the  $\beta$ -action: At low frequencies complete mechanical inhibition can be obtained without reduction of the e.p.p. This confirms observations by Marmont and Wiersma (9). The  $\beta$ -effect can sum at intervals of 0.1 sec.

3. Facilitation responsible for the growth of successive e.p.p.'s is not affected by inhibition at 50 per sec. The first or second e.p.p., after cessation of inhibitory impulses, reaches the amplitude to which it would have grown in the absence of inhibition.

4. It is suggested that the  $\alpha$ -effect is due to action of the inhibitor on the

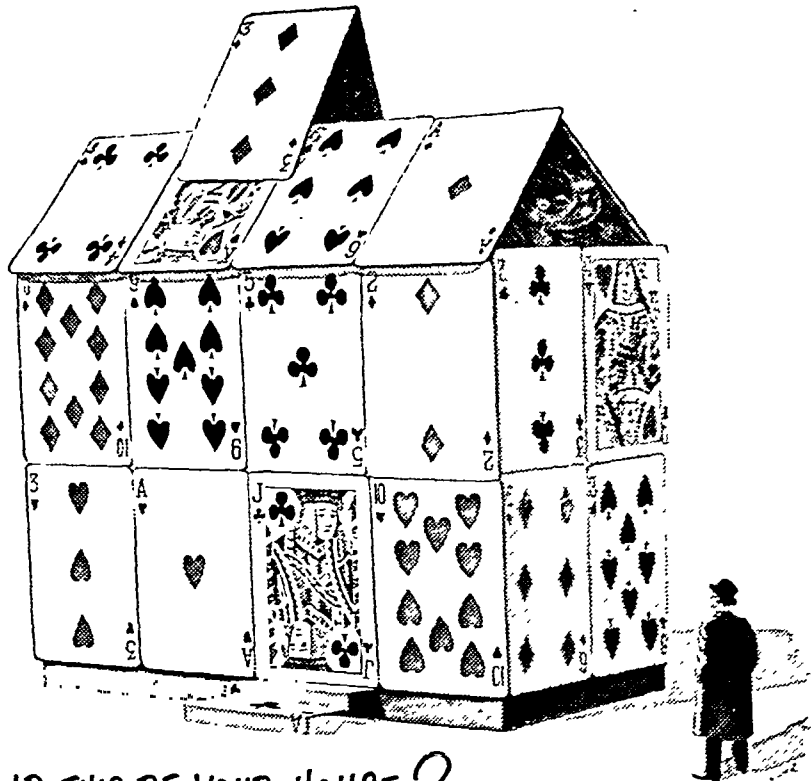
junctional receptors of the muscle membrane, and that the release of the transmitter is unaffected.

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# PRESENCE AND ACTION OF ACETYLCHOLINE IN EXPERIMENTAL BRAIN TRAUMA\*

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## INTRODUCTION

THE INADEQUACY of the various theories of cerebral concussion has been indicated by Denny-Brown in his recent review (14). In this review he also presented a systematic account of the recent evidence, both clinical and experimental, concerning the nature of this transient "traumatic paralysis" of the nervous system. In laying a firm foundation for the experimental approach to the problem of cerebral concussion, Denny-Brown and Russell (15) determined the physical conditions necessary for the production of graded injuries, and noted the alterations in neuronal function at and following the moment of injury. Observations on blood pressure, respiration and various reflex systems led them to conclude that moderate concussion results in transient suppression of the reflex motor activity of the brain. More severe concussion causes a complete and sometimes permanent paralysis of neuronal units.

Walker, Kollross and Case (59) studied the effects of concussion on animals (without barbiturates) and reported manifestations of an intense neuronal discharge at the moment of impact. These investigators concluded that post-traumatic depression is similar to those depressions which follow other forms of induced or spontaneous intense discharges of the central nervous system. A similarity between the post-traumatic and post-seizure phenomena had previously been recognized (19, 56).

The recent experiments of Groat, Magoun, Dey and Windle (24, 25) further elucidated the phenomenon of functional paralysis by demonstrating a sharp and immediate rise in supranuclear thresholds of excitation following impact. This elevated threshold might persist for relatively long periods following injury. Increased threshold in lower motor neurons became apparent as the severity of the concussion was increased. It appeared, further, that synaptic transmission is more affected by a mild concussion than is the functional activity of the neuron as a whole. It may be significant that, in spite of the general anaesthesia of chloroform, those neurons which increase the least in threshold of excitation—the lower motor neurons—showed on occasion a small, sharp and immediate decrease in their threshold of excitation. In this connection the excellent work of Windle, Groat and Fox (60) and Tedeschi (57) must be mentioned. Their histological studies have dis-

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closed that destructive changes may begin in brain tissue shortly after a concussive blow and may proceed for periods of time ranging up to months. A close relation has been established, moreover, between the severity of the blows and the severity of the subsequent anatomical alterations.

The present study was undertaken in an attempt to determine the part played by acetylcholine in severe forms of craniocerebral injury. The possibility of such a cholinergic effect is mentioned in the work of Babkin, Dworin and Schachter (3). The general hypothesis concerning acetylcholine (ACh), limited to the early post-traumatic condition in the present study, may be stated as follows: Under normal conditions, all the ACh released during neuronal activity is theoretically destroyed in the presence of the enzyme choline-esterase (ChE) concentrated at the cell membrane (44, 45, 46, 47). An abnormally intense or prolonged amount of neuronal activity causes the release of an abnormally large amount of ACh. In the abnormal condition, therefore, a part of the released ACh may escape destruction at the ChE barrier of the cell membrane and persist within the intercellular spaces to re-exert an abnormal effect on the surrounding tissue. That such an abnormal persistence of ACh might occur as a result of concussion or more severe forms of craniocerebral injury seemed possible from the previous demonstrations of Walker, Kollross and Case (59) and Lorente de N6 (37). The former, as already stated, described the intense neuronal discharge as a result of injury. The latter presented the equally important evidence that mechanically damaged cells of peripheral ganglia release ACh in estimable quantities without the necessity of stimulation.

If ACh were present in the intercellular spaces in a diffusible form, it might find its way into the cerebrospinal fluid (CSF). That evidence for the persistence of ACh might be found in the otherwise normal, *i.e.*, uneserinized, CSF was thought possible from the previous demonstrations of the relatively low concentration of ChE in that fluid (1, 4, 58), and ACh in the CSF might affect the brain from that locale.

The immediate problem, therefore, was to produce brain trauma in experimental animals (without barbiturates). For various periods of time following injury, the animals were to be studied for changes in behavioural and electroencephalographic (EEG) patterns. Samples of CSF were to be analysed for the presence of ACh at intervals following the production of trauma. If these analyses should prove positive, the effects of administered ACh would then be studied in an attempt to determine what action, if any, the presence of such amounts of ACh would have on the brain in comparison with the behavioural and EEG changes previously observed following trauma.

#### METHODS

For all major surgical procedures, adequate anaesthesia was induced by the intravenous or intraperitoneal administration of nembutal, 0.4 grain per Kg. body weight. On the occasion of head injury, dogs were under the influence of morphine sulphate, administered subcutaneously in doses of 1 grain per Kg. body weight, the dose not to exceed a total of 10 grains. Cats, subjected to experimental head injury, received 1 per cent

novocaine, locally infiltrated into the skin and muscle at the site of the blows. In those experiments in which ACh was perfused through a closed chamber overlying an area of exposed cortex, minimal amounts of nembutal, 0.3 grain per Kg., were used for the operative procedure of placing the perfusion chamber. The surrounding skin and muscle were also infiltrated with 1 per cent novocaine. In this way, surgical anaesthesia was obtained during the required period. When the EEG effects of nembutal had disappeared, the cat continued to lie quietly, permitting the experimental procedures to be carried out in an undisturbed sequence. (It should be noted that the greater the degree of barbiturate anaesthesia, the more difficult becomes the demonstration of the differential actions of ACh throughout the range of concentrations studied. Moreover, the scale of concentrations is displaced, so that higher amounts are needed to produce the effects which are evident at much lower concentrations in an animal which is not so deeply anaesthetized.)

Two methods of producing injury were used. Dogs and a few cats were submitted to a hydraulic blow transmitted directly to the brain through a column of water (59). This method is termed "compression concussion." In the majority of cases, "acceleration concussion" was produced by the forceful application of a weighted pendulum directly to the outside of the freely movable head (15). This latter method alone was used on the novocainized cats.

Following the injury, the animals were observed for reflex changes as well as for changes in more complex bodily conditions. At the time of the blow, the presence and duration of convulsive states were noted as well as the absence of ocular and corneal reflexes and the cessation of respiration. Subsequent depression or absence of hopping and placing reactions, the partial or complete loss of postural tone, sense of equilibrium and orientation were also noted. Examinations of cortical potentials (EEG) were carried out before, during and after trauma as the individual experimental conditions permitted. The standard condenser-coupled, push-pull amplifier recording with an ink-writing oscillograph with a range of frequency response between 0.5 and 50 cycles was used. The type of recording electrode varied, depending on the type of experiment. For the most part, vinylite-insulated phonograph needle electrodes were placed directly into the skull, the overlying scalp and muscle tissue having previously been infiltrated with 1 per cent novocaine. This type of electrode proved serviceable for either short or long periods of recording with the added advantage of easy insertion or removal during other experimental procedures. At times, lucite plugs, carrying a central silver electrode, were permanently embedded in previously prepared skull defects. All such recordings were bi-polar. In those experiments in which ACh was perfused over the exposed cortex, the outside of the duraluminum perfusion chamber was used as a reference lead in relation to an insulated silver electrode placed in the centre of the area being subjected to the effects of the perfusate.

All samples of CSF were withdrawn from the cisterna magna, cisternal puncture having been performed either under light ether or local novocaine anaesthesia. After withdrawal, the samples of CSF were diluted with frog Ringer-prostigmine (1:100,000) solution so that a sufficient quantity of fluid might be had for ACh determinations. The prostigmine was used to prevent the further action of small amounts of ChE which might normally be present in the CSF or the action of that ChE contained in the blood of those samples which were blood-tinged. All grossly bloody samples of CSF were valueless for ACh determinations and were discarded.

The ACh content of the diluted CSF samples was estimated by use of a prostigmine-sensitized frog rectus abdominis muscle bathed for 3 minutes in the fluid to be analysed. This method is sensitive to ACh in the concentration range of from 1.0 to 10.0 gamma per cent. A muscle preparation which did not show a relatively good sensitivity to 2.0 gamma per cent of ACh was discarded and another set up for the determination of ACh in the diluted CSF sample. Comparison of the contraction with known concentrations of ACh and calculations according to the original dilution of the CSF produced an estimate of the ACh content of the original fluid withdrawn. A number of samples demonstrating the presence of ACh by the response of the specific test object were submitted to hydrolysis studies for the further identification of the active substance as ACh. The samples were brought to about pH 9.0 by the addition of N/10 sodium hydroxide and were either boiled or permitted to remain at room temperature for about 24 hours. After hydrolysis, the samples were neutralized with N/10 HCl and retested.

Cholinergic and anti-cholinergic drugs were applied in the following manners: (i)

Varying concentrations of ACh, 0.5 to 10.0 gamma per cent in saline at body temperature, were perfused directly over an exposed area of cortex through a previously placed modified Forbes' window. (Extreme care was necessary during the preparation of the skull defect and dural reflection. The presence of slight amounts of blood or tissue exudate from muscle, bone, or injured cortical tissue might have allowed ChE to enter the field of perfusion and thereby have prevented the action of the minute amounts of ACh applied from an external source.) (ii) Small amounts of ACh (0.02 to 10.0 gamma in sterile saline) were injected into the cisterna magna after withdrawal of an equal quantity of CSF 0.5 to 1 cc. (iii) Atropine sulphate (in either 0.5 or 1 mg. per Kg. doses) was injected subcutaneously.

## RESULTS

The results to be presented fall into two main categories, the effects of trauma and the effects of administered ACh. A total of 182 experimental procedures on 18 dogs and 42 cats supply the results reported in the following series:

### *Effects of trauma*

Varying degrees of concussion were produced on 16 dogs and 27 cats. In all cases, the apparent indications of injury, as noted by previous workers, have been remarked. Tonic and clonic spasms lasting up to 1 minute after the blow have been observed as well as apnoea and the absence of ocular and corneal reflexes for as long as 2 minutes or more after the blow. These have been followed, in varying degrees, by slowing or absence of placing and hopping reactions, loss of body tone, sense of equilibrium, orientation, etc. These last effects have usually been accompanied by a stuporous state in which the animal was unresponsive to auditory, visual or other provocative stimuli.

The brains of traumatized animals presented in this study never showed gross damage or rupture, although petechial hemorrhages or rupture of a superficial blood vessel were sometimes seen. Histologically, a mildly degenerative disorganization of nuclear Nissl bodies was sometimes noted in brains of animals sacrificed from 3 to 30 days after trauma.

*Presence of ACh in CSF.* Including controls, 33 samples of CSF were withdrawn from the cisterna magna at various times following the production of trauma. Of these only those samples which were not frankly blood-filled were subjected to analysis for the presence of ACh. A total of 26 samples was so tested. In no instance did a control sample show any indication of the presence of ACh when tested on the frog rectus abdominis preparation.

In all but one case, which will be discussed, samples of clear or slightly blood-tinged CSF from traumatized animals caused contractions of the muscle preparation which, by comparison with known amounts of ACh, proved to have an original concentration varying between 2.7 and 9.0 gamma per cent. Moreover, the ACh content of the CSF persists in estimable quantities for periods of time ranging up to 48 hours following trauma. In two experiments on cats, the original concentrations of ACh in the CSF were 5.0 and 5.4 gamma per cent. Cerebrospinal fluid withdrawn from one animal after 29 hours had an ACh content of 3.7 gamma per cent. In 48 hours the ACh content had fallen to 3.0 and 3.1 gamma per cent after which time the concentrations of the diluted samples fell below the sensitivity of the muscle preparations.

Of the samples showing the presence of ACh, 7 were subjected to hydrolysis at pH 9.0 and all lost their ability to produce a contraction of the muscle preparation. The destruction of the active agent in the CSF samples and its identification as ACh is further demonstrated in the case of a dog from which 9 cc. of lightly blood-tinged CSF were obtained. The sample was divided into three equal parts, two of which were added to Ringer-prostigmine and the third to Ringer solution. The first two fractions had an estimated ACh concentration of 3.4 gamma per cent when first tested and lost their ability to produce a contraction of the muscle preparation after boiling at pH 9.0. The third fraction, in which the blood ChE was permitted to act, did not produce a contraction of the muscle preparation when first tested. Therefore hydrolysis in the presence of both hydroxide ion and the blood catalyst destroyed the active agent in the single sample.

Although the available data do not permit an absolute relationship to be shown between behavioural and EEG patterns as a measure of the severity of the concussion and the concentration of ACh in the CSF, there does appear to be a positive correlation between these two conditions. The one experiment which showed the least effects—no loss of consciousness, no spasms or apnoea, no change in behavioural pattern except for a general decrease in spontaneous activity—also showed no ACh in the CSF in spite of an extremely sensitive test preparation. On the other hand, maximal effects usually accompanied relatively high amounts of ACh in the CSF.

It appears, therefore, that ACh is liberated in abnormal amounts by traumatized nervous tissue and that some of the liberated ACh escapes destruction, persists in the intercellular spaces, and diffuses into the CSF where its presence may be determined. Moreover, the abnormal quantities of ACh persist for at least 48 hours in some cases, at which time the concentration falls below the sensitivity of the test object used in the present study.

*EEG in post-traumatic state.* The EEG changes previously reported were substantiated in this study. In cases where the experimental conditions were such as to permit recording during the time of the blow and immediately thereafter, an immediate, transitory excitatory discharge (59) was observed. Also, a later decrease in amplitude of all cortical activity and the consequent flattening of the EEG (15,18) was noted (Fig. 3C). The EEG changes to be described may continue for as long as 5 days. No permanent changes in the EEG were produced.

The following experiments were chosen as typical of the changes observed to occur. They are presented in the order of severity, the more severe first. In Exp. AC 72 (Fig. 1), a novocainized cat was subjected to maximum "acceleration concussion" resulting in short tonic spasms lasting from 1 to 6 seconds, and temporary apnoea followed by an absence of reflex reactions and a general stuporous condition. Electrographic recordings were then taken for 5 hours. On the following day, EEG records were taken for 10.5 hours.

Figure 1 illustrates the electrical activity of the cortex during these recordings. In 10 minutes continuous, high voltage 6-7 per second wave dis-

charges were recorded from both hemispheres, but the right hemisphere showed more continuous activity of a higher amplitude than the left. This type of activity gradually changed into paroxysmal rapid frequency discharges from both hemispheres, much in evidence 60 minutes after trauma. After 22 hours, however, the rapid frequency discharges were present only on the right and were almost continuous, tending to appear in spindles with a frequency of 16–20 per second.

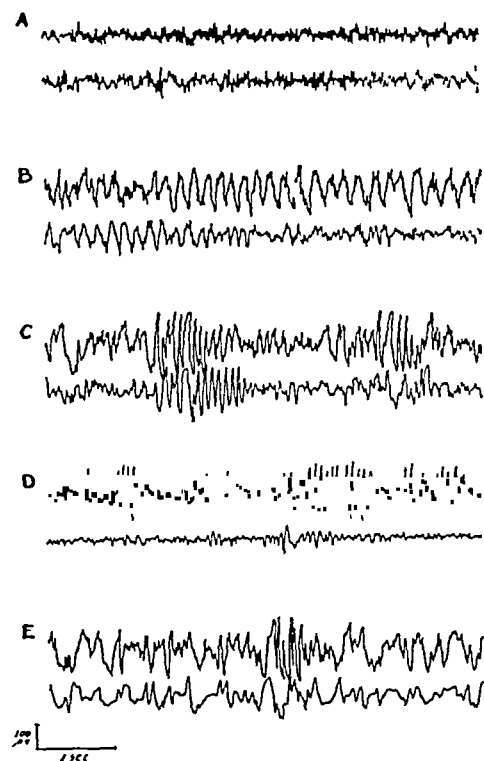


FIG. 1. Exp. AC 72. Cat. Maximum "acceleration concussion." Pen 1 right; pen 2 left (blows on right). A, control; B, 10 mins. after trauma; C, 60 mins. after trauma; D, 22 hrs. after trauma; E, 30 mins. after atropine, 22.5 hrs. after trauma.

For purposes of brevity, this type of high amplitude, rapid frequency discharge will be described as "epileptiform" although no suggestion of manifest convulsive phenomena is intended.

The lightly blood-tinged CSF, withdrawn 5.5 hours after injury, had an estimated ACh content of 7.0 gamma per cent.

Figure 2 is the record from a novocainized cat, AC 70, which had been subjected to a number of "acceleration concussive" blows, but which showed no obvious effects in behaviour or reflex patterns. There were present, however, persistent EEG abnormalities on the right, the side to which the blows were delivered. These consisted, for the most part, of continual "epileptiform" discharges. In the early period, the abnormal discharges were of rapid frequency, 17–20 per second, whereas later they slowed to about 10 per second. (Note the similarity between this record and that obtained 22 hours after a maximum effect, Fig. 1D.) The slower frequencies alternated with paroxysmal

bursts of the faster wave forms until the end of that particular day's recording. During all this time, the potentials from the opposite hemisphere preserved a normal appearance. Within 26 hours, the electrographic picture returned to normal and remained so.

This experiment is that in which no ACh could be demonstrated in the clear CSF although a sensitive preparation was used for the test. In view of the early disappearance of the EEG effect, the other data on ACh in the CSF, the similarity in type between the EEG changes in this experiment and those observed in more severe cases of head injury (as well as those EEG changes

which will be described as directly produced by cholinergic influences), it may be assumed that the observed effect was cholinergic, but that the ACh concentration was below the range of the test object. The high voltage rapid frequency discharges are similar in type to the activity seen during an epileptic seizure. Not only has this type of electrographic activity been present, however, but a fortuitous experiment bears out clinically characteristic features of the condition.

In Exp. AC 35, a dog, which was known to be predisposed to Jacksonian epileptic seizures, was submitted to the effects of a "compression concussion" while under the influence of morphine. Previously, the animal had been observed in two seizures which began in the right foreleg and spread in a characteristic manner to the rest of the body. Electrographically, there appeared a spike focus in the left hemisphere (Fig. 3A).

Immediately following impact produced by dropping a 400 gm. weight on to a column of water from a height of 6 feet, there occurred a tonic spasm of the head and upper trunk. This was unlike the pattern of its previous seizures. At the same time, respiration ceased and ocular reflexes were absent for 30 seconds. Within 15 minutes there ensued a prolonged period, over 2 hours, during which the dog had epileptic seizures similar in pattern to those previously observed. These seizures lasted from 1 to 3 minutes and were separated by 5 to 10 minute intervals. Thus a predisposition to epilepsy was precipitated into a series of focal seizures by a factor or factors introduced by a concussive blow.

Figure 3 shows the recorded electrical activity of the cortex. There was evident, first, the spike discharge from the left hemisphere and its early abolition, along with other types of normal cortical activity. This is the depression or flattening already described by others (15, 18, 59). Ten minutes later, however, the spike reappeared and developed until, 15 minutes after concus-

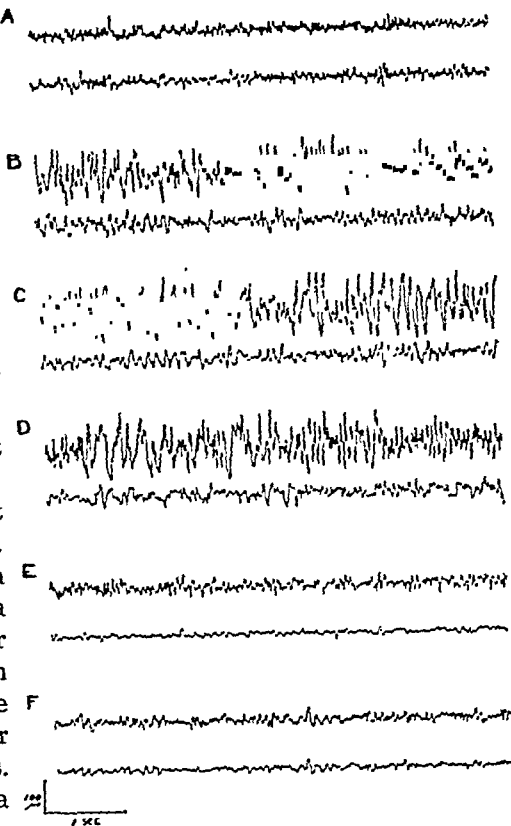


FIG. 2. Exp. AC 70. Cat. Sub-concussive effects. Pen 1, right; pen 2, left (blows on right). A, control; B, 5 mins. after trauma; C, 30 mins. after trauma; D, 2.5 hrs. after trauma; E, 26 hrs. after trauma; F, 42 hrs. after trauma.

sion, the first seizure occurred. Between seizures the cycle repeated itself—a flattened record, similar to that previously observed, the return of the spike, its development and its spread.

The evidence supplied by these experiments, therefore, indicates a persistent abnormality existing in the electrically recorded activity of one or

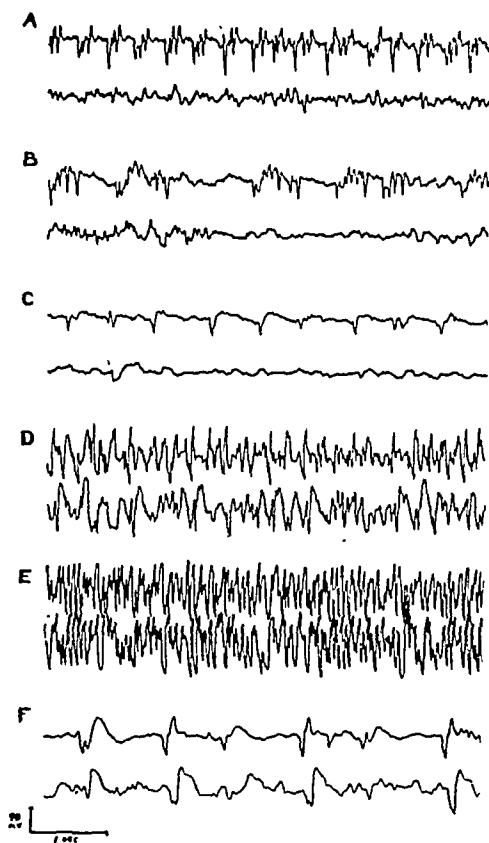


FIG. 3. Exp. AC 35. Dog. "Compression concussion" to morphinized dog predisposed to epilepsy. Pen 1, left; pen 2, right (blow on left). A, control; B, 20 secs. after trauma; C, 2 mins. after trauma; D, 30 mins. after trauma; E, 34 mins. after trauma; F, 37 mins. after trauma.

doses of 0.5 or 1 mg. per Kg. to a number of cats. The following two experiments were selected as indicative of the effects of this procedure.

As already described (Exp. AC 72, Fig. 1), there were "epileptiform" discharges recorded from one hemisphere 22 hours after a maximum concussion. At this time atropine, 1 mg. per Kg., acted to abolish the characteristic abnormality and to substitute instead random slow waves of irregular

both hemispheres of cats and dogs after the delivery of concussive blows. This abnormality, occurring at a later time than the previously described cortical phenomena, consists of high voltage discharges which appear in synchronized forms at frequencies varying from 6-7 per second to 17-20 per second. The slow rates appear soon after more severe concussion, later increasing in frequency, and the fast rates in milder cases. (This abnormality has been termed "epileptiform" for convenience.) Moreover, in the case of a dog predisposed to epileptic seizures, the abnormal discharge and its spread to other head regions were facilitated by, presumably, the same factors which induced the other related forms of EEG discharges.

*Effects of atropine on the post-traumatic state.* If the abnormal character of the electrical activity of the cortex and the behavioural pattern are due, at least in part, to the activity of abnormal concentrations of ACh within the brain tissue, atropine, an anticholinergic drug, should affect these phenomena when given in sufficient dosage. Atropine sulphate, therefore, was administered subcutaneously in

appearance. As shown in Figure 1E, the substitute abnormality appeared from the previously normal hemisphere as well. In the above example, atropine, administered after the full appearance and development of the EEG abnormality, acted to abolish it. In the following experiment atropine, administered within 30 minutes, acted to prevent its development. A cat, Exp. AC 74 (Fig. 4), was subjected to a similar procedure as in Exp. AC 72 with similar immediate results. Five minutes later the EEG consisted of high amplitude discharges at a frequency of 5-7 per second. Fifteen minutes later the frequency increased and the already familiar "epileptiform" discharges began to appear, primarily from the left. One-half hour after concussion, 1 mg. per Kg. of atropine sulphate was injected subcutaneously. Following the injection, the expected EEG changes failed to develop and, instead, the previously described random slow waves appeared from both hemispheres. At the same time, the cat recovered from its stuporous condition and assumed a sitting position. Although its appearance was greatly improved one hour following the atropine administration, no hopping or placing reactions could be elicited. The animal's alertness proceeded to the point where there appeared an excessive startle response to noise or movement. Five hours after injury, the EEG still showed occasional slow wave abnormality. The animal at this time was no longer hyperexcitable, but, attentive to stimuli, lay quietly. No hopping or placing reactions could as yet be elicited. The cat would unsuccessfully attempt to walk or run when placed on the floor.

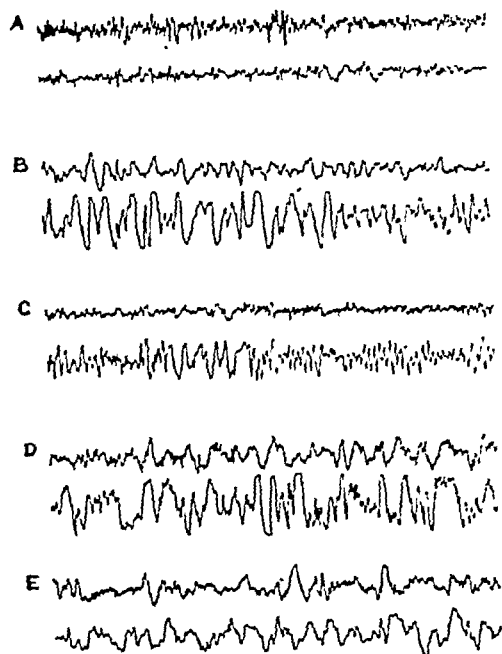


FIG. 4. Exp. AC 74. Cat. Atropine immediately after trauma. Pen 1, right; pen 2, left (blows on right). A, control; B, 5 mins. after trauma; C, 15 mins. after trauma; D, 37 mins. after atropine, 1 hr. after trauma; E, 1 hr. 37 mins. after atropine, 2 hrs. after trauma.

These experiments indicate, therefore, that atropine in sufficient dosage not only abolishes the characteristic EEG abnormality but also prevents its development. This drug also counteracts stupor and loss of body tone but does not appear to affect the loss of co-ordinated hopping and placing reactions. These results thus suggest that certain of the characteristic changes noted in the function of the nervous system are due to the presence of abnormal quantities of ACh and its action on the surrounding tissue.



*Effects of ACh applied to the brain*

ACh is found persistently present in the CSF of dogs and cats following the application of concussive blows. At the same time the electrical activity of one or both hemispheres of the cortex as well as the external behaviour of the animals show patterned changes. The question arises concerning the possibility of reproducing these changes by the application to the central nervous system of ACh within the range of concentrations present following trauma.

*Effects of perfusing ACh directly over the cerebral cortex.* In general it may be stated that the recorded electrical activity of a cortical area over which ACh (in saline at body temperature) is being perfused is dependent upon the concentration of ACh in the perfusate. Small physiological concentrations excite or synchronize, whereas large amounts depress the electrically recorded cortical activity of dogs and cats. The effects of 1.0 to 4.0 gamma per cent ACh are shown in Figure 5, the record from a lightly nembutalized cat. The application of 1.0 gamma per cent ACh produced high amplitude slow and sharp wave discharges. On the other hand, 2.0 and 4.0 gamma per cent caused a marked flattening of the record.

On replacing higher concentrations of ACh with saline after the effects of the higher concentration had been produced and during the return to the control level, *i.e.*, while passing through a stage of decreasing cholinergic influence, large amplitude waves were again evident. The higher concentrations sometimes produced rhythmical low amplitude slow waves as an accompaniment to the flattened record. It was difficult to decide whether this was an effect produced by the ACh as such or merely a pre-existing underlying rhythm uncovered by the suppression of the normal, more rapid frequencies. Finally, it was demonstrated that the effects of smaller concentrations can be abolished by higher concentrations when the varying amounts are applied in succession.

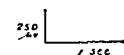


FIG. 5. Exp. AC 94. Cat. Perfusion of ACh over cortex of lightly nembutalized cat. Concentric recording. Right temporal. A, control; B, 1.0 gamma per cent ACh; C, 2.0 gamma per cent ACh; D, during recovery from 2.0 gamma per cent ACh; E, 4.0 gamma per cent ACh.

plitude waves were again evident. The higher concentrations sometimes produced rhythmical low amplitude slow waves as an accompaniment to the flattened record. It was difficult to decide whether this was an effect produced by the ACh as such or merely a pre-existing underlying rhythm uncovered by the suppression of the normal, more rapid frequencies. Finally, it was demonstrated that the effects of smaller concentrations can be abolished by higher concentrations when the varying amounts are applied in succession.

*Effects of ACh injected intracisternally.* Since an intracisternal injection

might be accomplished with a minimal disturbance to the experimental animal, after local novocainization of the injection site, this method of the application of ACh to the brain was preferred to that of perfusion. Also, without barbiturates, observations could be made on the behavioural changes as well as on the changes of the otherwise normal activity of the cortex, as recorded with the EEG. In these 12 experiments, ACh was injected in amounts ranging from 0.02 to 10.0 gamma. Inasmuch as the diffusion gradient for ACh was from tissue to CSF in the first experiments, and since in the later experiments the gradient was reversed, it was thought that the amounts administered lay well within the range of concentration previously found. Although the intracisternal injection of saline caused no observable effects on either the behavioural or EEG patterns, ACh produced marked changes in both.

Soon after the injection of 10.0 gamma of ACh in Exp. AC 95 (Fig. 6), the cat became very quiet and listless. At the same time, occasional paroxysmal "epileptiform" discharges with a frequency of 10-18 per second appeared in the EEG. These discharges did not continue in this manner, however, but gradually decreased in amplitude and frequency. The decrease in recorded cortical potential proceeded beyond the original control record so that

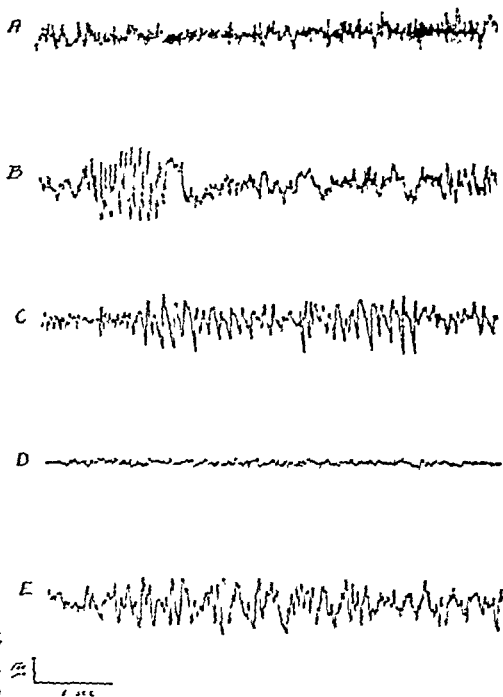


FIG. 6. Exp. AC 95. Cat. Intracisternal injection of 10 gamma per cent ACh. Bipolar recording from right parieto-central region. A, control; B, 6 mins. after injection; C, 22 mins. after injection; D, 43 mins. after injection; E, 2.5 hrs. after injection.

finally a marked flattening of the EEG occurred, attaining a maximum about 45 minutes after the ACh injection. By that time, the cat had become stuporous and unresponsive to external provocative stimuli. Although this external depressed state continued to be present, the cortical rhythms gradually returned to the previous abnormal "epileptiform" patterns and continued until the end of the recording period 3 hours later.

Following the injection of smaller amounts of ACh, such as is shown in Exp. AC 91 (Fig. 7) in which 2.0 gamma of ACh was injected, similar abnormalities appeared except for the absence of the intervening flattened record. The EEG showed a marked increase in the amplitude and frequency of cortical discharges, with the appearance of "epileptiform" patterns, which

continued for varying periods of time proportional to the amount of ACh applied. In 4 experiments, atropine sulphate was injected subcutaneously in doses of 0.5 to 1 mg. per Kg. during the period of cholinergic activity. Within one half hour after the injection (Fig. 7E), the characteristic paroxysmal EEG abnormality was abolished and was replaced by the already familiar random slow wave discharge. The injection also invariably brought

about a recovery from the stuporous condition induced by the previous ACh injection.

It is evident, therefore, that ACh in low physiological concentrations, when perfused over the cortex, produces excitatory or synchronizing effects. Depressant effects appear when the concentration attains a value of 2.0 gamma per cent or higher. The same process may be demonstrated in experiments in which ACh in varying amounts is injected into the cisterna magna. In these latter studies, the EEG and behavioural changes are strikingly similar to those seen following trauma of varying intensities and, like them, can be abolished by the injection of adequate amounts of atropine.

#### DISCUSSION

Although denied by some workers (16, 21), there are previous reports (12, 43) that ACh may be found in increased quantities in the CSF following experimentally induced nervous activity. It is worth

noting, moreover, that ACh has been found present in the CSF of patients who had various neurological and psychical illnesses (48). The ACh shown to be present in the CSF following the delivery of concussive blows to dogs and cats is presumably that which has escaped destruction at the ChE barrier of the cell membrane (44, 46, 47). Although the ACh is now extracellular and out of the sphere of the normal intracellular mechanisms of nerve impulse transmission (45), it does appear capable of exerting an effect on nervous tissue either to depress or enhance activity, depending upon its concentration. The concept of qualitative alteration in the effect of varying amounts of ACh (i.e., that high physiologi-

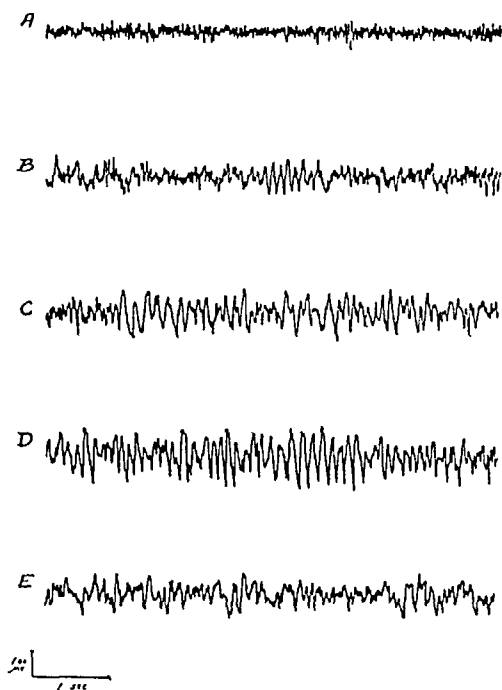


FIG. 7. Exp. AC 91. Cat. Intracisternal injection of 2.0 gamma per cent ACh. Bipolar recording from right parieto-central region. A, control; B, 6 mins. after injection; C, 17 mins. after injection; D, 20 mins. after injection; E, 53 mins. after injection and 15 mins. after atropine.

cal concentrations depress, whereas low concentrations enhance or excite neuronal activity) is contained in many previous studies of the neuromuscular junction (10, 26, 42, 54) and peripheral or central ganglia (5, 6, 7, 10, 22, 23, 38, 40, 51). Moreover, in the course of their research various investigators (17, 27, 31, 32, 53) have reported persistent drowsiness or "sleep" following the intrathecal administration of cholinergic drugs to animals and man. For purposes of discussion, therefore, as well as for differentiation from the intracellular ACh released during normal neuronal activity and rapidly destroyed, the name "free ACh" will be applied to that which is persistently present extracellularly, is capable of diffusion, and is free to act on the surrounding nervous tissue. Also, in corroboration of previous studies of cholinergic and anti-cholinergic factors within the CNS (11, 22, 33, 40, 41, 43), "free ACh" is also blocked by appropriate doses of atropine, although such action has been denied (16, 31, 52, 55).

In discussing the role of "free ACh" and its possible relationship to the demonstrated changes following trauma, it is necessary to distinguish between the conditions existing within the CNS, as recorded electrographically, and those in the external behaviour of the animal. A central excitatory or "hyper-synchronous state" may be manifest externally as much by signs generally thought to be depressed in character as by an external demonstration of excitation, as in the unconsciousness attending petit mal and grand mal discharges—both characterized by an enforced activation or synchronization of otherwise discretely discharging groups of neurons. The existence, therefore, of high amplitude activity of various frequencies in the EEG of experimental animals following trauma or the application of ACh is not inconsistent with a coincident state characterized by stupor and apathy to visual, auditory, or other stimuli.

It has been shown that the transient depressed or flat type of EEG (15, 18, 59) may be duplicated by the application of high physiological amounts of ACh within the range of "free ACh" found in the CSF following trauma. Earlier studies (41) have also shown such a flattening when ACh was applied to the cortex without the previous application of eserine. These and similar studies (8, 13), however, are difficult to relate to the present problem because of the great discrepancy in the doses of ACh used. Jasper (28) concluded from the then available evidence that ACh in small doses has a facilitating effect, whereas large doses have a depressing action on the electrical activity of the cerebral cortex. It is suggested, therefore, that "free ACh" is a factor in the production of the depressed activity of the cortex, as evidenced in the flat EEG, following trauma to the brain.

In the light of the important demonstrations (15, 24) that in the majority of cases concussive blows produce an abolition of reflex activity and an increase in the excitation threshold of motor neurons, the question arises of the possible role "free ACh" may play in causing these alterations.

In his review, Brown (9) describes a number of experiments proving that large quantities of ACh, released by repetitive stimulation or applied from

an external source, may paralyse the ganglion cells, either partially or completely. More recent studies of reflex activity of the spinal cord in the cat (52), the dog (11, 38) and in man (32) confirm this fact. Similar results have been obtained for the respiratory centre (22, 23). Moreover, in direct studies of thresholds of excitation of the motor cortex of cats, intracarotid ACh produced a depression or obliteration of the response, particularly with larger doses (43).

It was stated in the introduction that this paper attempts to present a general hypothesis concerning "free ACh" and excessive neuronal activity, and early data tend to substantiate the presence of "free ACh" as a result of other experimental and clinical conditions. Thus, preliminary data indicate "free ACh" following electrically induced seizures in both dogs and man as well as following clinical epileptic attacks. On the basis of what is known as the "extinction" phenomena (20), the detailed studies (39) on ten human epileptics appear relevant. In general, the effects of an induced seizure closely parallel the data presented by the experimental studies of cortical electrograms and thresholds of stimulation following head injury (24, 25). Previous work tends to show that ACh in high physiological concentrations has a tendency to cause a paralysis of synaptic activity and to depress neuronal excitability.

Since "free ACh" accompanies the post-concussive EEG and behavioural patterns which may be reproduced by the intracisternal application of quantitatively similar amounts of ACh and since these changes may be partially abolished by sufficient doses of atropine in both cases, it is suggested that "free ACh" is a contributing factor in causing the transient "traumatic paralysis" of cerebral concussion. That it is not the only factor involved is evident from a number of considerations: (i) blood, with its attendant ChE, is often found in the CSF although no gross damage to brain tissue may be demonstrated; (ii) although the stuporous state and the EEG patterns were abolished by atropine, reflex alterations were not appreciably affected. These and other chronic post-traumatic phenomena may be due to the histological changes now known to occur (57, 60). Moreover, other physiological factors, such as potassium ion, may also play a part in the production of early post-traumatic functional alterations.

There are also early post-traumatic phenomena which are not due to a "traumatic paralysis" of the nervous system. Muscular hyperactivity—even in the presence of unconsciousness or stupor (30)—and the tendency to epilepsy or pre-epileptic states in the early post-traumatic period (59) have been noted. (The primary concern here is not with the late development of epilepsy, the subject of considerable study, which has been shown by Penfield and his associates (49, 50) to be due, in part at least, to the presence of meningo-cerebral cicatrices.) Indeed, previous experimental evidence on concussion also demonstrates excitatory changes. Thus, in moderate concussion, motor centres of the brain may have been stimulated although reflex activity was paralysed (15). Also, in the study on thresholds of excitation (24) a

decrease in the threshold was sometimes noted in those neurons which were most resistant to concussive effects.

It is not surprising, therefore, that previous epileptoid tendencies (as in the case of the dog described) or a new source of abnormal discharge (as may be produced by local injury) may be increased in strength and likelihood of spread by the favorable environment for synchronous activity. It is pertinent to note that human EEG records of early post-traumatic epilepsy (29) are similar to those seen in the present study both in the form of "epileptiform" patterns and during actual post-traumatic seizures. Jasper and Penfield further suggest a neuronal hyper-irritability as one physiological factor in the appearance of these as well as in the more chronic convulsive discharges. In this connection, the recent demonstrations of a spreading cortical depression (with occasionally occurring repetitive high amplitude discharges) following light mechanical or electrical stimulation of the rabbit's cortex appear significant (34, 35, 36). Although unstated amounts of ACh were applied to the cortex in an attempt to simulate these phenomena and no correlation could be found, it may be significant that potassium ion, whose synergistic action with ACh is well known, could reproduce the effects obtained by electrical or mechanical stimulation. It is a possibility that "free ACh" in small abnormal amounts is a contributing factor in the production of early post-traumatic excitatory states.

"Free ACh" having been demonstrated as a possible extracellular factor in one abnormal condition, the question arises whether it is a factor in other abnormal states following excessive neuronal activity, and also whether it is a qualitative or quantitative variation from the normal. If quantitative, what is the rôle of pre-existing "free ACh" in normal amounts on the known phenomena of normal neuronal activity and what effects are produced by disturbances in related enzymatic and metabolic processes?

#### SUMMARY AND CONCLUSIONS

1. As a result of experimental trauma to the head, ACh is consistently present in the CSF in estimable quantities, 2.7 to 9.0 gamma per cent within a few hours of injury. The abnormal amounts of ACh may be detected for as long as 48 hours following trauma, after which time the concentration falls below the sensitivity of the test object. The abnormal presence of ACh is presumed to be due to an excess production or release of the substance, an insufficient destruction, and consequent persistence within the intercellular spaces. It has been suggested that such persistent ACh be termed "free ACh."

2. The EEG of a number of cats and dogs have been studied for varying periods of time following trauma. Confirmation for both the previously described intense neuronal discharge and the transient flattening of all recorded electrical activity has been obtained. Following these effects, there occurs a prolonged period of abnormality in one or both hemispheres. The

abnormalities are essentially paroxysmal, high amplitude sharp waves with frequencies varying from 6-7 per second to 16-20 per second.

3. Changes in behaviour include tonic-clonic seizures, apnoea, and loss of ocular and corneal reflexes followed by partial or complete loss of hopping and placing reactions, sense of equilibrium, orientation, and a stuporous condition for varying periods of time of from hours to days.

4. The EEG patterns and the stuporous condition may be abolished by appropriate doses of atropine sulphate.

5. ACh perfused over an exposed area of cortex produces high amplitude sharp waves in small physiological concentrations, 1 gamma per cent or less, and a flattening of recorded cortical potentials in high physiological concentrations, 2 gamma per cent or more (depending upon the depth of general anaesthesia).

6. The intracisternal injection of ACh in amounts ranging from 0.02 to 10.0 gamma produces similar behavioural and EEG changes as previously noted, *i.e.*, transient flattening with high concentrations and paroxysmal, high amplitude sharp waves of varying frequencies with low concentrations.

7. The EEG and behavioural effects of intracisternal ACh may also be abolished with appropriate doses of atropine sulphate.

8. It is suggested that "free ACh" may be one of the physiological factors underlying the acute paralytic and excitatory phenomena of cerebral concussion and more severe craniocerebral injuries.

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# THE RELATION OF ELECTRIC POTENTIAL CHANGES TO CONTRACTURE IN SKELETAL MUSCLE

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## INTRODUCTION

FOR THE PURPOSE of this study contracture may be defined as a prolonged, reversible, not conducted contraction of a muscle. The extensive literature on this subject has been surveyed by Gasser (12) in a critical and comprehensive review. The aim of the present investigation was to record the electrical potential changes which accompany contracture, and to study the role which the muscle membrane plays in initiating and maintaining the processes of contraction. Potentials during contractures have already been recorded by Biedermann (2) and other workers and more recently by Bremer (3). In investigations on the whole muscle, however, it is difficult to record accurately from the site of the contractures. Further, it is not always possible to distinguish electrically propagated from local contractions when they occur simultaneously. Such difficulties have now been largely overcome by using completely isolated muscle fibres.

Contractures were produced by constant current pulses and by chemical stimulation. A number of chemical substances available in the laboratory, representing compounds of very different chemical properties, were tried. Whenever active they seemed to produce responses of essentially the same kind. In most experiments, however, only acetylcholine, nicotine, potassium and veratrine were used. The action of these four drugs when applied to muscle has already been studied in detail in isolated muscle fibres (21,22). The first three drugs are substances which can produce contraction and contracture directly on application while the veratrine effect appears only after a propagated response has been set up.

## METHOD

Potential changes which occur during contractures can be most accurately followed in single muscle fibres. These were obtained from the *M. adductor longus* of *Hyla aurea*, and if the preparations are carefully dissected the shunting effect of fibre rests and saline is small and muscle spikes of 60 to 100 mV are recorded with the preparation suspended in paraffin oil. Local responses which may be a small fraction of propagated impulses can be easily detected. The majority of experiments, however, were performed on preparations containing 2-4 muscle fibres. These preparations were easier to handle, they stood up to frequent drug applications, "washing," lifting through the saline-paraffin interface, and generally stayed in good condition for several hours, giving normal fully propagated responses whenever stimulated directly or through their nerve. Placing of electrodes and the

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general recording technique has been described in previous papers (21, 22). Thus potential changes produced by drugs like potassium or acetylcholine (ACh) were recorded during application to one of the recording electrodes (21), while the veratrine effect could be conveniently recorded after any form of muscle excitation (22). Drug application or constant currents which produced contractures did not appear to injure the muscle fibres. Contractures could be set up repeatedly in the same preparation over periods of many hours.

Amplifier distortion, when recording long-lasting potential changes, was appreciable, a rectangular voltage input falling to half in three seconds. In the present experiments, however, special attention will be paid only to the earlier part of the potential changes, while it will still be possible to calculate the approximate value of potentials after 3-4 seconds. When ACh was applied to muscle fibres, the innervation of the preparation was preserved so that the drug could be placed on the neuromuscular junction where it produced potential changes in very small concentrations.

Contractures of isolated fibres were observed directly under the microscope. Their occurrence is unmistakable and it is always possible to distinguish with certainty a local shortening from a propagated muscle response. Contractures set up in sartorius muscles by prolonged constant currents or drugs were recorded isometrically on a torsion wire myograph connected to a kymograph. Deflexions could also be registered through an optical system attached to the myograph.

## RESULTS

*Contractures after veratrine application.* Veratrine produces a large negative potential following propagated muscle impulses, and at a critical threshold level this potential may initiate further muscle discharges (22). The veratrine-tetanus may be followed by a contracture which can be clearly observed under the microscope in isolated fibre preparations. Membrane changes leading up to and accompanying contractures are illustrated in Figure 1, on three different preparations consisting of two muscle fibres. A drop of veratrine in concentration of  $10^{-6}$  and  $10^{-5}$  was applied to the tip of one of the wick-electrodes. Contractions and contractures arose under the veratrine-electrode after a muscle impulse has passed over that area (22). In Figure 1A a single stimulation of the two fibres sets up a short tetanus of high frequency (nearly 300 per sec. in Fig. 1Ab). While one fibre ceases to propagate abruptly after a series of discharges, the other continues to show small responses at the height of the negative potential. These responses, however, do not reach the second electrode, as is recognized from the absence of the diphasic wave. Their number varies greatly in different muscles and even in the same preparation. The exposures in Figure 1Aa and b were taken at an interval of about half a minute. Close examination of Figure 1Ab revealed approximately twelve of these non-propagated responses of about the same frequency as the previous discharges. Not fully propagated (abortive, local) impulses are presumably accompanied by local activity of the contractile elements (see Discussion). A succession of such responses would, therefore, represent a prolonged local contraction. Abortive impulses are usually observed at the end of a long series of discharges, or after a few and occasionally even after a single propagated impulse. They are always followed by a negative smooth potential change which may be maintained for several seconds. At the same time contractures were observed, lasting for approximately the same periods as the smooth negative potential changes. Accurate determinations, however, of the temporal relationship between negative potentials and contractures could not be made in this investigation.

As each impulse in a series of muscle responses in veratrinized muscle is set up by a negative afterpotential one may suppose that the subsequent contracture would also be initiated by a similar event in the muscle membrane as will be shown in the second and third Sections. It is, therefore, of interest to observe the gradual transition (well seen in Fig. 1Ab) between the potentials giving rise to contraction and contractures.

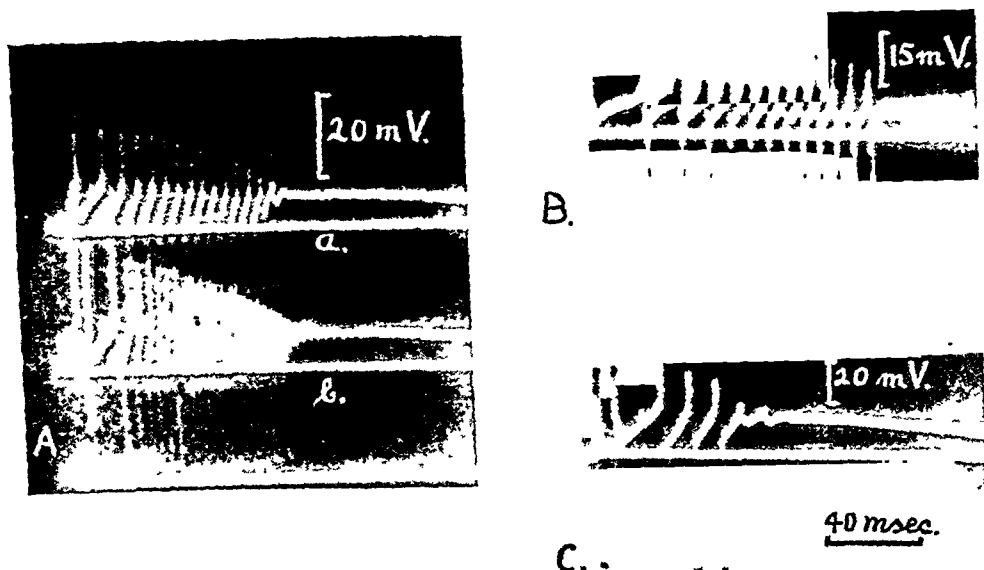


FIG. 1. Electric potential changes recorded from three different preparations containing two muscle fibres. A small drop of veratrine (concentration  $10^{-3}$  and  $10^{-5}$ ) was applied to the region in contact with one recording electrode. Then the muscle fibres were electrically stimulated and the impulses passing over the veratrinized region initiated a series of discharges which were recorded at the site of their origin. A: a and b recorded at an interval of half a minute. The two muscle fibres are discharging almost simultaneously. One fibre ceases to discharge abruptly while the other fibre continues to set up small responses which do not reach the second recording electrode. The following smooth negative potential may persist for several seconds. B: Note the gradual decrease of the muscle spike height in the veratrine region while the diphasic component remains constant. C: The first two potentials are set up by stimulation of the muscle fibres at an interval of 8 msec. At the veratrine region they initiate three fully propagated responses and some "abortive" impulses.

*Contractures in response to direct drug application.* Contractures following application of drugs have been studied by many workers (cf. 12). Langley (23) investigated the effect of nicotine when applied in droplets to muscles. He obtained three types of responses: (i) tetanus; (ii) tetanus with subsequent contracture; (iii) contracture alone. Similar experiments were performed on isolated fibres in the present investigation, but in addition electrical potentials were recorded. Besides nicotine, acetylcholine (ACh) and potassium were also used. These drugs cause a depolarization of the muscle

membrane directly on application (21). Riesser and Steinhausen (28) have already shown that the contractures set up by ACh were associated with a negative potential.

The initiation of chemical contractures by membrane depolarization could be demonstrated in experiments as illustrated in Figure 2b. A drop of a Ringer solution containing 0.6 per cent KCl was applied to two muscle fibres. An immediate contracture resulted, lasting for several seconds; this was

accompanied by a negative potential which was maintained for approximately the same time. With reduction of the KCl concentration, a diminished membrane depolarization followed drug application; and at concentrations usually ranging between 0.2 and 0.4 per cent KCl, no visible sign of contractures could be detected. Further reduction of KCl, far below threshold concentration, still set up a progressively diminished negative potential change. It therefore appears that contractures, like twitch-like contractions, arise, when a sufficient membrane depolarization is reached. While, however, thresholds for twitch-like responses can easily be observed, no accurate detection of the contracture threshold is possible, as no method is available to register the very small local muscle shortenings.

ACh or nicotine have the same effect as KCl when applied to the end-plate regions. Contractures not preceded by propagated responses were regarded as a sign of "fatigue" after prolonged electrical stimulation or drug action. Often preparations recovered from such a state of "fatigue" and responded with propagated impulses to the same drug-concentration after washing in saline for 10-30 minutes.

Figure 2a illustrates the type of potential changes which generally accompany drug application in "fresh" preparations. There the depolarization potential leads up to a series of muscle impulses which are followed by several small, not fully propagated, responses leading up to a maintained large negative potential. Comparison with records of Figure 1 shows a similar sequence of potential changes when twitches and contractures are set up in veratrinized muscles. Also during the exposure of Figure 2a a twitch was observed, followed by a contracture.

During contractures initiated by direct drug application or arising at veratrinized regions, propagated impulses set up elsewhere on the muscle

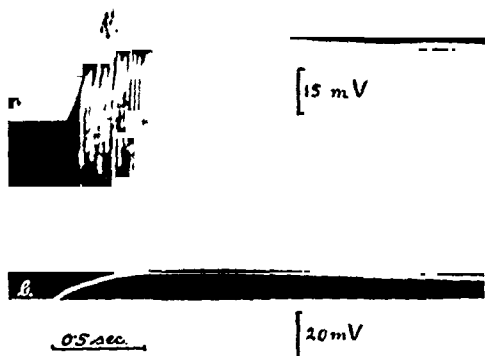


FIG. 2. *a*: Recording from the region of local application of  $10^{-5}$  ACh to several muscle fibres. Note the small local responses following on the propagated muscle impulses. The maintained negative potential is accompanied by contracture. *b*: Potential change in two muscle fibres set up by 0.6 per cent KCl, producing contracture. (The apparent potential decay is due to the relatively short time constant of the amplifier.)

fibres could not pass the contracture. The likely reason for the blocking of a propagated impulse seems to be the state of the membrane which has lost its property to conduct in the contracture region. This muscle portion would then constitute a block which may perhaps be overcome if the contracture were confined to a very small area of the fibre. The situation is different when a *subthreshold* potential, like an endplate potential, is in the path of a muscle impulse. Then the impulse approaching that region is speeded up (cf. 8, Fig. 9; 20, Fig. 7). Depression or abolition of the responses of muscles during contracture has been described by Dale and Gasser (7) and G. L. Brown (5).

*Inhibition of contractures by constant current pulses.* The production of contractions and contractures by constant currents has already been studied in great detail by Biedermann (2). It is well known that a sufficiently strong constant current pulse initiates a series of contractions at the cathode. If the muscle is repeatedly excited or otherwise fatigued, a prolonged contracture follows on the tetanic response and after further stimulation contractures alone may be set up at the cathode. These contractures are maintained during the passage of the current and their magnitude is dependent on the current strength (cf. 24). It was frequently observed that relatively fresh muscles reacting with contractures to prolonged current pulses still gave twitch-like propagated responses when excited with induction shocks. The similarity between the action of constant currents and drugs is very striking. Contractions or contractures alone, or together, can be set up by both methods. Also the membrane changes accompanying drug application or current pulses at the cathode, prior to or following initiation of muscle impulses, are comparable (20, Fig. 10; 21, Fig. 2).

The "cathodic shortening" which takes place during current flow is clearly set up and maintained by depolarization of the muscle membrane. If the chemically produced contracture is similarly set up it should be possible to inhibit it at the anode while a constant current is applied. Relaxation of contracture at the anode of an applied current has been found in the case of veratrine contractures by Biedermann (2). This was confirmed in the present experiments and relaxation was also observed for chemical contractures set up by drugs which excite "directly" on application (Second Section).

Contractures were generally produced around one electrode by local application of potassium or acetylcholine. These contractures were observed visually through a microscope and were also recorded myographically with a light isometric lever. They could be maintained for periods varying from several seconds to one or two minutes, depending on the quantity and concentration of the applied drug. If a constant current was passed through such a preparation with the anode at the region of the contracture, relaxation of a great part of the contracted region around the electrode was invariably observed. The extent of the relaxation depended greatly on the current strength. The minimal current intensity to set up a contracture at the cathode was determined in several experiments. The same current gen-

erally effected a perceptible relaxation at the anode if passed through a region at which a drug-contraction had been set up. During these experiments one stimulating electrode was usually placed on the tibial section of the muscle while the other was near the pelvic end which had been previously crushed or "killed" by heating. This prevented stimulation of the pelvic end and thus myographic records showed only the tension or relaxation produced by current leaving or entering the muscle membrane in the area around one electrode. Otherwise, contractions which developed under the cathode obscured the myographic registration of relaxation occurring at the same time at the anode. The tension set up by drug application and afterwards reduced at the anode during current flow was usually restored nearly to its former level on breaking the current. As would be expected, cathodal currents augmented the contractions.

*Effect of novocaine on contractions.* It is well known that application of novocaine or of other anaesthetics abolishes propagated muscle responses, while strong induction shocks cause local twitch-like responses only. It is still possible, however, to set up contractions with long current pulses (12). These findings were readily confirmed when the muscles were bathed in 0.1 to 0.5 per cent novocaine hydrochloride dissolved in Ringer. The threshold at which contractions were set up by constant currents was determined in several fatigued but otherwise normal muscles. After bathing the preparation for 10 to 20 minutes in novocaine-Ringer solutions the contraction thresholds were not raised. They were actually lowered in many observations. At the same time the muscles were practically inexcitable with short induction shocks. Due to variations of electrode and muscle resistance when changing the fluid bath, however, small threshold alterations could not be reliably measured. Similarly, the potassium threshold was not appreciably affected and 0.2 to 0.3 per cent KCl which normally sets up propagated impulses at the point of application gave rise to small contractions in novocaine-treated muscles. When the accompanying potential changes were recorded in isolated fibres, a negative potential similar to that of Figure 2b was observed. Novocaine, however, greatly depressed the acetylcholine and nicotine sensitivity of the endplates (27). In this respect it acts similarly to curarine.

The resting potential of regions to which novocaine (0.1–0.5 per cent) was applied locally showed no marked change. Also the time course of the polarization potential of the muscle membrane was investigated in these preparations by the method of Katz (18). The muscle was inserted into a direct current bridge and constant current pulses were balanced at various moments of current flow. The resulting electrotonic potentials, recorded from the bridge output, were not altered appreciably in shape and size by novocaine concentrations of 0.1 to 0.5 per cent. This indicates that the electric time constant of the muscle membrane was not significantly changed. It has not been possible to determine the membrane characteristics which are changed by novocaine and which thus prevent the conduction of impulses. It is clear, however, that the depolarizing action of long-lasting currents or of drugs is not affected,

Some observations were made on the reduction of the resting potential and of the electric time constant of the normal muscle membrane by potassium. In muscles bathed in Ringer containing an excess of potassium (0.2 to 0.6 per cent) the decay of the electrotonic potential became progressively quicker with increase of the potassium concentration. At the same time the electrotonic potential itself was reduced in size. These findings are in agreement with many observations made on the change of the electric time constant in muscle or nerve during reduction of the resting potential (17, 18).

The raised threshold for short shocks after novocaine is probably only apparent. Since normal conducted responses are prevented, the threshold is determined by the appearance of a different type of contraction which seems to last for the duration of an applied current. These local contractions may still be obtained at normal current intensity, but can only be seen if the applied current lasts long enough for the externally developed tension or shortening to become perceptible.

### DISCUSSION

It was found in this investigation that contractures are accompanied by a negative potential change. In fatigued, injured, or narcotized muscles direct evidence shows that the negative potential precedes and sets up the contractures. Accordingly a weak concentration of a drug may cause on direct application a small negative potential only, without activating the contractile mechanism. On increasing the drug concentration the negative potential may attain a sufficient size to cause a contracture (Second Section). In "fresh" muscles the contractures usually follow a prolonged tetanus and are likewise accompanied by a negative potential. Like those not preceded by a tetanus, such contractures can be suppressed at the anode of an applied constant current. Furthermore, frequently the whole cycle of transition from propagated to abortive (local) impulses and to the subsequent maintained negative potential could be shown in single muscle fibres. The gradual transition is especially well seen in veratrine-treated muscle (Fig. 1) although the mechanism seems essentially similar when "directly" depolarizing drugs are applied. There is no evidence for any fundamentally different processes appearing between the local spike-like response and the subsequent smooth negative potential change. The contracture seems to be set up in the same way as the preceding fully or partially propagated muscle impulses. The muscle membrane, however, gradually loses during the tetanus its capacity to conduct the membrane changes which then stay confined to a small region. Such contractures are frequently set up at a similar threshold level at which the preceding propagated or "abortive" impulses were initiated (Figs. 1 and 2). Changes in the muscle responses are therefore merely due to gradual alterations in the condition of the muscle membrane. Incomplete restitution of the normal membrane resting potential at the end of a tetanus or after prolonged experimentation may be the cause of the "fatigue" which favors the development of contractures. Thus, a muscle which responds to a certain concentration of a drug with contractures only,



may afterwards give prolonged discharges when the same amount of the drug is applied after rinsing or washing of the fibres.

By analogy with other known evidence obtained from nerve and muscle, it seems that contractures are set up and maintained by a local depolarization which is produced by applied currents or by the depolarizing action of a drug (Third Section).

Neuromuscular contractures have been observed in eserinizid muscles by Cowan (6) and their associated potential changes were recorded by Feng (10, 11). It is now clear that those contractures were maintained by the prolonged depolarizing action of the endplate potentials (9).\*

Bremer's findings (3) that potentials seem to precede the first development of tension is in good agreement with the present results. Our preparations, however, do not give contractures which persist for an appreciable time after cessation of stimulation. It seems likely that in those muscles the membrane potential persisted after withdrawal of a stimulus, especially since the contractures could also be inhibited at the anode (see later; also 13).

It was stated by some investigators that the membrane changes occurring during contractures were due to chemical processes associated with the contractile mechanism. This view seems unlikely for the following reasons: (i) Experiments show that similar membrane changes, such as occur during contractures, are also seen with subthreshold concentrations of veratrine or other drugs. Only on reaching a certain threshold intensity are contractures set up. Accurate contracture thresholds, however, cannot be determined as the visual or myographic registration of the least response is not very critical. But it is still possible that processes in the contractile elements *contribute* to the negative potential during contracture. (ii) Analogous potentials occur in veratrinized nerve, obviously originating in the membrane (1, 16). (iii) A similar sequence of events is observed in muscle at the cathode of an applied current pulse. There the membrane changes which eventually set up contraction or contracture appear *immediately* on current application (20). (iv) Inhibition at the anode suggests a primary membrane effect (see below).

Much work has been done in recent years on the conditions which determine the different types of responses in muscle. Especially the experiments on single muscle fibers have given much valuable new information (4, 13, 14, 15, 25, 26, 29, 30). On the whole, the extensive work in this field was not concerned with the initiation of muscle activity by membrane changes. A study on the correlation between potential changes and mechanical responses is still lacking, presumably because of the difficulties involved in recording changes other than propagated responses. It seems clear, however, that the graded responses which can be obtained by gradually increasing current

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\* Recently non-propagated neuromuscular contractions were found in normal frog muscles. These are set up on stimulation of small-diameter nerve fibres in contrast to the well-known fully propagated responses set up by nerve fibres of larger diameter. (Kuffler, *Proc. Soc. exp. Biol.*, N. Y., Sept. 1946, in press.)

strength or drug concentrations are primarily determined by the extent of the membrane changes.

Similar observations were made on crustacean muscles in which local neuromuscular contractions occur normally. In these preparations the contraction rate and magnitude can be accurately controlled by varying the rate and number of nerve stimuli, which in turn determine the local potentials which cause the local muscle activity (19). The extent of the membrane changes when graded contractions are set up by stimulation of muscle fibres with microelectrodes (13, 14, 15) is not clear. The membrane area involved in those experiments may not be large enough to set up propagated impulses and it may not be possible to detect the local potentials without recording from the stimulating cathode and the site of the local graded responses (20, Fig. 11).

There is at present no indication concerning the nature of the link between muscle membrane and contractile elements. It seems, however, that action currents flowing during depolarization do not play an essential role in the "excitation" of the contractile system. For instance, in a muscle which is entirely immersed into a contracture-producing solution, the whole membrane will be simultaneously depolarized. It is clear that in such cases no current flow from the "normal" to a depolarized region can occur. Such contractures covering a large area, or those produced over a few mm. as in Figure 2 will be inhibited at the anode of a constant current pulse. It seems, then, that a drug like KCl or ACh produces a contracture by its action of removing at least part of the membrane potential. Any process restoring the membrane to its original polarized state will then cause a relaxation of a contracture. On withdrawal of the anodal current the depolarizing effect of the drug, if still there, will reassert its action and again set up the contracture (Third Section).

Since it appears that there is no essential difference in the setting up of twitch-contractions and contractures, it would also follow that the currents of a propagating muscle impulse have no direct excitatory effect on the contractile elements.

#### SUMMARY

Experiments were performed on nerve-muscle fibre preparations of the M. adductor longus and on whole isolated sartorius muscles of frogs (*Hyla aurea*). Contractures were set up by constant current pulses and by application of drugs.

1. Negative potential changes are always recorded at the site of origin of contractures in isolated muscle fibres.

2. Contractures, like propagated muscle responses, are initiated, after a sufficient depolarization of the muscle membrane. Contractures may arise: (i) following on muscle impulses which may gradually fail to propagate fully from the region of their origin. In these preparations a transition can be detected from normal to "abortive" impulses and to a maintained negative potential change which may give rise to contractures without appreciably

exceeding the potential level at which the preceding propagated responses had been set up; (ii) not preceded by propagated responses following on the depolarizing action of drugs or currents in fatigued, narcotized or injured muscles.

3. Contractures set up by chemical application are actively maintained by the depolarizing action of drugs. This action is analogous to the "cathodic shortening" effect which lasts for the duration of the current flow. "Chemical" or "electrical" contractures can be graded, depending on drug concentration or current strength.

4. Relaxation of chemically produced contractures can be effected at the anode of constant currents.

5. Novocaine does not raise the threshold at which contractures are set up by constant currents or by potassium application. The apparent threshold for brief shocks, however, is greatly increased. The electric time constant and the resting potential of the muscle membrane is not significantly affected.

6. The connection between the muscle membrane and the contractile elements is discussed. It is suggested that the action currents which accompany depolarization or the propagated muscle impulse are not the essential link in the transmission of "excitation" from the membrane to the contractile elements.

#### ACKNOWLEDGMENT

I wish to thank Dr. B. Katz for much helpful discussion in the course of this investigation.

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## ERRATUM

Eccles, Katz and Kuffler, *J. Neurophysiol.*, 1942, 5: 220. The two paragraphs, 'If a burst of repetitive volleys . . . general discussion below,' should be inserted on page 219 before the small print section headed 'Neuromuscular facilitation after eserine'.

# RIGHTING AND OTHER POSTURAL ACTIVITY IN LOW-DECEREBRATE AND IN SPINAL CATS AFTER D-AMPHETAMINE<sup>1</sup>

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WHILE OBSERVING the influence of d-amphetamine upon the muscular coordination of acute preparations of low-decerebrate and of spinal cats, we noted some unusual postural behavior suggestive of righting. The present report describes this and other postural activity after d-amphetamine. Our observations indicate that there are centers for righting in the pons, medulla, and spinal cord in addition to the generally accepted centers in the mid-brain.

## METHODS

These observations were made upon 27 low-decerebrate cats and upon 20 cats with complete sections of the spinal cord at levels between C6 and T8. The operations, which are described in some detail below, were performed under ether anesthesia. Observations were not begun until at least an hour after cessation of ether inhalation and were limited to the first ten hours following the operation. Body temperature was maintained near the normal level by means of a heating pad. D-amphetamine sulfate<sup>2</sup> was injected intraperitoneally, usually in a dose of 10 mg./kg.

*Decerebration.* Both common carotid arteries were ligated. From a trephine hole in the parietal bone the dura overlying most of the cerebral hemisphere of one side was exposed. The dura was opened and the brain-stem was completely sectioned with a blunt spatula in a plane beginning dorsally at the tentorium and ending ventrally caudad to the optic chiasma. Ether inhalation was immediately discontinued. The skull overlying the opposite cerebral hemisphere was then quickly rongeué away and the brain rostrad to the section removed. A second section of the brain-stem was now made caudad to the pituitary stalk, exposing the posterior clinoids. All the brain rostrad to this final section was removed. Compression of the vertebral arteries which has been commonly practiced by others was not found necessary at any time during the operation for the performance of a relatively bloodless decerebration. The skull was loosely filled with absorbent cotton.

At the end of each experiment, the remaining brain was removed and preserved in 4 per cent formaldehyde. In the decerebrate cats, the brain-stem was cut by a plane passing through the following landmarks: dorsally, either between the colliculi or through the inferior colliculus, and ventrally, either at or caudad to the place where the oculomotor nerve leaves the brain-stem. In some cats, the plane of section lay dorsally through the caudal part of the inferior colliculus and ventrally through the rostral part of the pons. The anterior pole of the cerebellum was not touched and the superior cerebellar artery which supplies the anterior cerebellum was usually intact. Shortly after the completion of the decerebration, these animals usually showed vigorous reflexes elicitable from the cornea, pinna, vibrissae, tongue, and jaws. The presence of these reflexes indicated that the cranial nerves V through XII were intact and functioning.

*Preparation of the spinal cats.* The spinal cord was exposed and completely sectioned at the desired level (between C6 and T8). The spinal section was followed by decerebration. Ether inhalation was then discontinued. Observations were limited to those parts of the body which were caudad to the spinal section.

<sup>1</sup> This work was supported by a grant from the Milton Fund and Clark bequest.

<sup>2</sup> Kindly supplied by Smith, Kline and French Labs., Philadelphia.

## RESULTS

*Time-course.* A number of characteristic changes occurred gradually over a period of from 5 to 60 minutes after the intraperitoneal injection of 10 mg./kg. of d-amphetamine sulfate in cats which had been decerebrated one or more hours previously. When the animal was allowed to lie quietly on its side, the first evidences of change were in the respiratory system. The rate and volume of respiration began to increase within about 5 minutes of the injection. The respiration also became more irregular, and sighs occurred frequently. The frequency of spontaneous swallowing increased.

Shortly after the first increase in respiration, the following peripheral effects of d-amphetamine were noted in both the decerebrate and spinal cat: marked pilomotor erection most noticeable over the tail and back, widening of the palpebral fissures, retraction of the nictitating membranes, further dilation of the already large pupils, and salivation. The repeated swallowing which is one of the earliest signs of the syndrome brought on by d-amphetamine may well depend upon the increased salivary flow resulting from this drug. In several animals, copious mucus escaped from the anus at intervals during the action of this drug.

Righting activity and other changes in postural behavior first became detectable about 15 minutes after the injection of d-amphetamine sulfate. The syndrome reached its peak of development in from 30 to 40 minutes after the injection and remained constant thereafter for up to 3 hours.

*Decerebrate rigidity.* In these experiments, extensor rigidity was usually not marked in the low-decerebrate cat at the time of the injection (Fig. 1A), except in a few cats in which the superior cerebellar artery was damaged during the operation. When the animal lay on one side, the neck was not hyperextended. The legs were moderately stretched out, the claws were sheathed, and the paws were slightly flexed. Extensor tone was usually more marked in the forelegs than in the hindlegs. The tail possessed tone, and when placed in extremes of position it returned somewhat toward mid-position. The tip of the tail twitched slightly from time to time. The labyrinthine limb reflexes were minimal except in the presence of marked decerebrate rigidity.

After d-amphetamine, the extensor rigidity was unchanged or even diminished in many cats, especially in those which showed marked righting activity. In some cats, however, the extensor rigidity was accentuated after d-amphetamine. Twitching of the tail increased.

When the body was supported from beneath at the upper part of the chest and at the lower part of the abdomen, the head and tail were consistently supported higher after d-amphetamine than before the drug was given. Both went to mid-position and rarely achieved the extreme hyperextension sometimes seen in decerebrate rigidity.

*Righting activity.* An acute low-decerebrate cat does not make any attempt to lift its head when it is laid on its side, providing that it is not under the influence of any drug (Fig. 1A). Approximately 15 minutes after the

intraperitoneal injection of d-amphetamine sulfate, however, some decerebrate cats slowly lifted their heads (Fig. 1, B-F). The typical decerebrate cat lifted its head from the door-mat upon which it was lying and turned it so that the eyes were level. The shoulders were raised from the door-mat by a lateral flexion of the upper back and neck. The forelegs assumed a position which tended further to right the shoulders. The upper forepaw was flexed at the wrist and the distal part of the foreleg was rotated so that the upper paw faced downward. Accompanying this there was some flexion of the upper

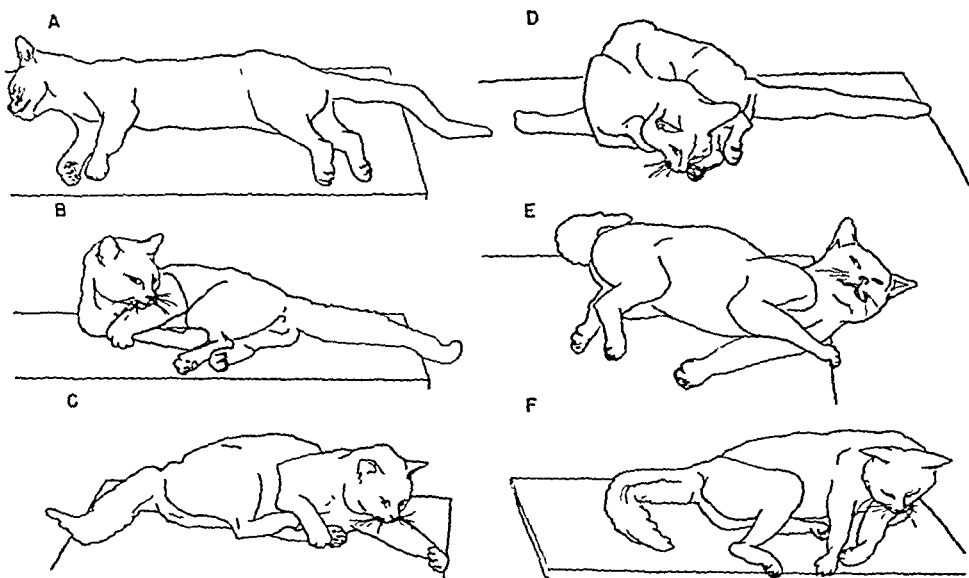


FIG. 1. Righting activity of low-decerebrate cats after d-amphetamine. A: Typical posture of low-decerebrate cats placed in side position before d-amphetamine. B to F: Typical postures achieved by different low-decerebrate cats after having been placed in side position after d-amphetamine.

forelimb. The upper forepaw thus tended to grasp whatever surface or edge might be within its reach and to pull the shoulders toward an upright position by flexion of the leg. The animals which reached this stage did not usually succeed in getting the lower elbow under the trunk, but remained in the half-righted position seen in Figure 1, with head level and shoulders high.

The grasping activity of the upper forepaw and foreleg just described tended to occur in both forepaws when the animal was not in side position. The flexion at wrist, elbow, and shoulder was frequently accompanied by spreading of the toes and baring of the claws. In some decerebrate cats treated with d-amphetamine, this activity was so marked that the cat could be suspended by its forepaws from the edge of a chair-back or a table for 10



to 30 seconds. These animals all showed marked righting activity when laid in side position.

In some cats, the righting of the head and shoulders from side position was accompanied by similar but less marked activity in the rump, hindlegs, and tail (Fig. 1F). The rump and tail were elevated from the table. The hindlegs were flexed and assumed positions which furthered righting of the rump.

The righting activity of low-decerebrate cats was accentuated by contact of the cat's side with the table after a period of support in the air, by contact of the cat's side with a rough surface such as a fiber door-mat, by slight shaking of the door-mat upon which the cat was lying, by stimulation of the toes or of the perineal region, or by acceleration of the cat upward or downward while in mid-air in side position. Especially with acceleration, the tail underwent rotary motions similar to those in intact cats righting themselves while falling freely in air (7). The direction of the rotation depended upon the side to which the cat's body was inclined. Thus if one observed the cat from behind, the rotation was clockwise when the animal was lying on its left side, counterclockwise when the cat was lying on its right side—*i.e.*, the motion of the tail was dorsal in the ascending phase of the rotation and ventral in the descending phase.

If one placed a board or one's hand lightly upon the upper side of a decerebrate cat which had lifted its head from the table, the cat slowly lowered its head until it again rested on the table.

Some cats lifted their heads if placed in the side position on one side, but not if placed on the other side. Many of the cats, however, lifted their heads and exhibited other righting activity when lying on either side.

There was a negative correlation between the degree of decerebrate rigidity and righting activity after d-amphetamine. Those cats which showed extreme rigidity before d-amphetamine showed slight or no righting activity after d-amphetamine. Low-decerebrate cats which originally showed only slight extensor tone were the most apt to show righting activity after d-amphetamine.

A change of posture sometimes noted as a result of d-amphetamine in the decerebrate animal lying on its side, and possibly related to righting activity, was a slight arching of the back and a change in the position of the legs so that the forepaws and the hindpaws were close together. This change varied considerably in degree from cat to cat.

Of 20 cats with complete sections of their spinal cords at levels between C6 and T8, 11 showed rotary motions of the tail after d-amphetamine when lying on one side and when the lateral aspect of the hindknee which was next to the table was rubbed. These rotary motions were similar to those which occurred in low-decerebrate cats during acceleration upward or downward while in mid-air in side position. Eight other spinal cats lifted their tails from the table during this rubbing. Ten of the 20 spinal cats responded to this rubbing with incomplete righting of the rump accomplished by move-

ments of the hindlegs and elevation of the lumbar spines from the table.

*Standing of the decerebrate cat.* When the body was supported from beneath at the upper part of the chest and the lower part of the abdomen, the cats without d-amphetamine showed a posture typical of a moderate degree of decerebrate rigidity. The legs were extended, the tips of the toes (rather than the toe-pads) being the lowest parts; the head and tail were partly supported, and the back was flat. If the animal was now lowered, the paws made feeble, if any, response to contact with the table. If the positions of the feet were stable, weight bearing often occurred for short periods, the animal gradually sinking to a couchant position.

When these tests were performed in animals under the influence of d-amphetamine, distinct changes were evident. When extensor rigidity was increased, the forelegs were especially hyperextended in this position. The forepaws were usually facing caudad, so that the tips of the toes were the first to meet the table. The response of the forefeet when they met the table was not improved by d-amphetamine. By contrast, the hindpaws faced obliquely between horizontal and vertical, the toes were partly extended, and the claws were usually bared. When the hindpaws touched the table, there was frequently a response of the foot characterized by further extension and spreading so that the toe-pads met the table. When the extensor activity of the hindlegs was marked, this either did not occur at all or resulted in an awkward, tip-toe position.

After d-amphetamine, the hindlegs supported the animal's weight more steadily and for a longer period of time than before the drug. If the forepaws were placed on the table and all of the legs were placed in a wide stance at suitable positions, standing usually occurred with only slight support of the neck or no support at all. In some of the decerebrate animals after d-amphetamine standing was interfered with by a turning of the head and body to one particular side, by marked arching of the back and opposition of the fore- and hindpaws, or by active stepping of the hindlegs (next section).

*Stepping in decerebrate and spinal cats.* We tested some of our animals for stepping in the following manner: The animal was supported in a standing position in such a way that the toe-pads of all four legs, or of the fore- or hindlegs alone, were resting on the table. The body was then moved forward. There was no response to this forward displacement of the body in animals before the administration of d-amphetamine. After d-amphetamine, however, alternate progressive stepping movements of the hindlegs occurred in response to this movement of the body in 6 out of 11 low-decerebrate cats tested and in 2 out of 6 spinal cats tested. This stepping consisted of a lifting of a hindleg high under the body and then extension of the leg and placing of the paw on the table in a good standing position some distance ahead of its former position. This was followed by similar stepping of the opposite leg. The extended leg usually supported the hindpart of the body adequately. If the forepart of the body was supported under the chest, one could sometimes induce the amphetamine-treated decerebrate or spinal cat to walk across the

table on its hindlegs. We were unable to obtain stepping with the forelegs. One possible explanation of our failure is that the amphetamine-treated decerebrate cat tends to flex its forepaws continuously (Fig. 1), thus making it more difficult to achieve a standing position of the forelegs than of the hindlegs.

*Placing.* No placing of the forepaws was observed in any of our cats. Placing of the hindpaws was produced in 4 out of 11 low-decerebrate cats after d-amphetamine as follows: The animal was supported and the ankle was flexed by pressure of the edge of the table against the distal part of the leg. After a few seconds, there often occurred a typical placing response with flexion of the leg, the foot being moved slowly and gracefully upward and forward, with toes outspread, and lowered to meet the table. Placing of the hindpaws did not occur in 6 spinal cats tested for this response.

### DISCUSSION

We have studied the influence upon the behavior of decerebrate and spinal cats of d-amphetamine, a substance known to affect the intact central nervous system in a striking manner. We found that this substance generally increases the activity of the portions of the nervous system which remain in these animals. It may be recalled that ephedrine, a close chemical relative of amphetamine, increases the activity of spinal animals. One might argue that the increased activity observed after the administration of d-amphetamine resulted from an improvement in the circulatory status of the experimental animals. Yet throughout long experiments (8 to 10 hours) the animals had regular respiration and active reflexes whether or not they had received d-amphetamine; and they appeared to be in excellent condition at the time that d-amphetamine was injected. It should be emphasized that the "spinal animals" described in this paper had normal control of the respiratory and circulatory systems, since the forepart of the animal was in the decerebrate state, the observations being made on the hindpart nervously isolated by the section of the spinal cord.

None of the reflexes or forms of spontaneous activity observed were diminished by d-amphetamine. Some of the reflexes, however, were not appreciably affected. Righting, stepping, and placing may be regarded as postural activities increased by d-amphetamine. By contrast, standing, another type of postural activity, was not markedly improved in the decerebrate cat. In this instance it is possible that the increased spontaneity of movement produced by the substance interfered with the steady muscular activity necessary for standing. Similarly, there was a mutual antagonism between righting activity and decerebrate rigidity; when one was prominent, the other was interfered with.

In cats acutely decerebrated by a section low in the mid-brain, righting activity is eliminated (6, 8). Hence the righting centers have usually been placed in the brain-stem above this plane of section. By the use of d-amphetamine, we have found that righting activity occurs in cats in which

he section excludes the mid-brain. The phenomena described in this paper were observed in animals in which the plane of section lay dorsally through the caudal part of the inferior colliculus and ventrally through the rostral part of the pons. This section excludes the substantia nigra, the red nuclei, and the nuclei of the oculomotor nerves. We therefore conclude that centers for righting exist caudad to this section, *i.e.*, in the pons, medulla, or spinal cord. Even in the acute spinal animal, we have observed righting activity of the rump, hindlegs and tail. Hence we further conclude that spinal centers for these activities exist.

Asymmetrical body contact appears to be the most important stimulus for the righting activity which we have observed. In the decerebrate cats no special effort was made to exclude the influence of the labyrinths. Yet the weight of a board on the upper side of the body of a decerebrated cat which had righted its head made the animal slowly return its head to side position. In spinal cats the importance of asymmetrical body contact is emphasized by the absence of labyrinthine influence and by the fact that additional cutaneous stimulation on the side next the table was necessary in order to bring on the postural activity.

A comparison of the results described above in acute decerebrate cats under the influence of d-amphetamine with the activity of chronic decerebrate cats as studied by Macht (4) reveals many similarities. Macht's animals with sections of the brain-stem at the level of the exit of the oculomotor nerve from the brain-stem exhibited righting activity not unlike that of our animals. The stepping observed after d-amphetamine both in decerebrate and in spinal cats is closely related to the proprioceptive hopping reactions described by Macht in chronic decerebrate cats (see also 1 and 9). The placing of the hindlimbs which we observed is similar to that of Macht's cats. Both are proprioceptive responses and should not be confused with the tactile placing reactions described by Rademaker (9) and by Bard (1). The righting and grasping activity of the forelimb of our decerebrate cats is similar to that observed by Macht. A similar reflex pattern has been observed in decorticate and thalamic monkeys (3, 5). Macht's chronic decerebrate cats apparently had less righting activity of the hindquarters than we have described. His three cats with the lowest transections (in the rostral part of the pons) did not spontaneously right either the fore- or the hindquarters as did our animals with comparable transections. Yet even our spinal cats under the influence of d-amphetamine had righting activity of the hindquarters. On the whole, the similarities between Macht's data and our own are more striking than the differences. In terms of righting activity, d-amphetamine appears to have about the same effect upon the lower righting centers as does survival for a period of days or weeks. Acute decerebration presumably removes the discharges from higher centers which keep these lower centers active; having at first no activity of their own, the lower centers do not reveal themselves in the behavior of the acute decerebrate animal; but when d-amphetamine is introduced or the passage of time per-

mits the centers to develop a spontaneous activity, they again influence posture.

### SUMMARY

Righting and other postural activity was observed in low-decerebrate cats and in spinal cats after the intraperitoneal injection of d-amphetamine sulfate, usually in a dose of 10 mg./kg. In the decerebrate cat, the righting activity consisted of elevation of the head and shoulders from the surface upon which the cat was lying and of movements of the fore- and hindlegs, rump, and tail which resulted in incomplete righting of the body. The tail rotated in a manner suitable to promote righting. In spinal cats similar righting movements were observed in the hindlegs, rump, and tail.

Asymmetry of body contacts is essential for righting activity in the decerebrate cat under the influence of d-amphetamine. In spinal cats after d-amphetamine, righting activity does not appear unless there is asymmetry of body contacts plus additional tactile stimulation of the lateral aspect of the hindknee which is next to the table.

Our data indicate that, in addition to the previously known centers in the mid-brain, there are centers for righting caudad to the mid-brain, *i.e.*, in the pons, medulla, and even the spinal cord.

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# STIMULATION WITH MINIMUM POWER

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VARIED wave-forms have been used for stimulating nerve and muscle, including sine waves, exponentially falling currents, inductive impulses (effectively a sum of exponentials), and square waves. Except where differential stimulation between several fibres is desired, the optimum wave-form is that which stimulates the fibre with least injury. Although the exact relationship between current, time, and damage has not been determined, it is probably proportional to the heat generated:

$$H = a \int_0^{t_0} I^2 dt \quad (1)$$

$I$ , the current, is applied from time  $t=0$  to  $t=t_0$ ; and  $a$  is a constant. While accommodation (6) or inhibition (5) must be considered for long sustained currents, for brief currents a single first order differential equation describes the excitatory process:

$$dE/dt = KI - kE \quad (2)$$

and excitation occurs when  $E = E_0$ .

Integration of (2) gives the condition for excitation to be

$$E_0 = Ke^{-kt_0} \int_0^{t_0} Ie^{kt} dt. \quad (3)$$

The optimum wave-form of current  $I(t)$  will therefore, under the above assumption, be that which makes (1) a minimum, subject to condition (3) for excitation in time  $t_0$ .

Using the method of the calculus of variations (2), the desired wave-form is determined:

$$2I + \lambda Ke^{-kt_0} e^{kt} = 0$$

$$I = -\frac{1}{2} \lambda Ke^{-kt_0} e^{kt} \quad (4)$$

where  $\lambda$  is a constant "undetermined multiplier": Thus the desired current is an exponentially increasing wave. If  $\lambda$  is eliminated between (4) and (3),

$$I = 2kE_0 e^{kt} / K(e^{kt_0} - e^{-kt_0}). \quad (5)$$

Putting (5) in (1) gives for the heat generated:

$$H = 2akE_0^2 / K^2 (1 - e^{-kt_0}).$$

It is seen that the heat is a minimum when  $t_0$  is infinite, and equal to

$$H_{\min} = 2akE_0^2 / K^2,$$

and the current is

$$I_{\min} = 2kE_0e^{kt}/K \quad (6)$$

where the zero of time is taken at  $E = E_0$ .

Thus the wave-form stimulating with minimum power is an exponentially rising current (a positive exponential).

For a constant current, integration of (3) gives the current required to excite at  $t_0$  to be

$$I = kE_0/K(1 - e^{-kt_0}), \quad (7)$$

and the heat generated is

$$H = ak^2E_0^2t_0/K^2(1 - e^{-kt_0})^2. \quad (8)$$

To minimize (8), the variable part is differentiated with respect to  $t_0$  and put equal to zero:

$$\begin{aligned} 0 &= (1 - e^{-kt_0})^2 - 2t_0k(1 - e^{-kt_0})e^{kt_0} \\ e^{kt_0} &= 1 + 2kt_0 \\ t_0 &= 1.257/k \\ H &= 2.44 akE_0^2/K^2. \end{aligned} \quad (9)$$

Thus a square wave requires 22 per cent more power to excite than the best current form.

The rheobasic current is found from (7) by making  $t_0$  infinite:

$$I_{\text{rheo}} = kE_0/K$$

and the chronaxie is the excitation time with twice this current:

$$\begin{aligned} 2kE_0/K &= kE_0/K(1 - e^{-kt_0}) \\ t_{\text{chron.}} &= 0.69/k. \end{aligned}$$

Thus the duration of a square wave of optimum intensity must be 1.82 times the chronaxie to excite.

The exponentially rising current was found to be optimum. Many stimulators are, however, made using exponentially falling currents. This is the wave-form produced by the well known thyatron stimulator circuits. The power required to stimulate with the best wave of this type will be determined. The exponentially falling current is

$$I = I_0e^{-bt} \quad (10)$$

where the current is applied from  $t=0$  to  $t=\infty$ , and  $I_0$  is the initial value of the current. In a thyatron stimulator,  $1/b$  is the RC product of the timing circuit.

Putting (10) in (2), and integrating, noting  $E=0$  when  $t=0$ ,

$$E = KI_0(e^{-bt} - e^{-kt})/(k - b) \quad (11)$$

$E$  rises to a maximum,  $E_{\max}$ , at some time  $t_{\max}$ , and then falls.  $t_{\max}$  is found by differentiating (11), and setting  $dE/dt=0$ , to be

$$t_{\max} = \log (b/k)/(b - k). \quad (12)$$

Putting (12) in (11) gives

$$E_{\max} = (KI_0/k)(b/k)^{b/(k-b)}.$$

For excitation,  $E_{\max} = E_0$ , and

$$I_0 = (kE_0/K)(b/k)^{b/(b-k)}. \quad (13)$$

Putting (13) and (10) in (1) gives the heat generated with the exponentially falling current:

$$H = aI_0^2/2b = (aE_0^2/2K^2)b^{(b+k)/(b-k)}/k^{ek/(b-k)}.$$

The minimum value is found by differentiating with respect to  $b$ , and setting the derivative equal to zero. This gives the minimum at

$$b = k.$$

Excitation occurs at time  $t = k$ , and the minimum value of heat is

$$H = \frac{1}{2}e^2 \cdot akE_0^2/K^2 = 3.694akE_0^2/K^2.$$

Thus the most efficient exponentially falling current requires about 85 per cent more power to stimulate than the optimum exponentially rising current, and 51 per cent more than the optimum square wave.

### DISCUSSION

While the exponentially rising current is apparently the most efficient in stimulating nerve (at least when accommodation is neglected), such currents are not readily produced by simple electronic circuits. The currents which are most readily generated with accurately controllable wave-form are square waves, exponentially falling currents, and sine waves. Of these the first is the most efficient, requiring only 22 per cent more power than the best wave-form. The second is somewhat less efficient, requiring 85 per cent more power.

The analysis of sine waves is complicated by several factors: the wave-form is a function of the repetition rate; anelectrotonus and catelectrotonus occur during recovery; and with waves as slow as 60 cycles, accommodation cannot be neglected. Actually, sine wave stimulation will require several times the power required by any of the other wave-forms discussed (4).

It thus appears that unless a different wave-form is required for some special purpose such as differential stimulation, square waves should be used for stimulation of nerve or muscle. The duration of the square waves should be approximately 1.26 times the time-constant of excitation, i.e., 1.82 times the chronaxie. While it may not be essential to use the least possible power in laboratory stimulation of nerve, in clinical work—and especially in electric



shock therapy—it is very desirable to do so in order to minimize damage to tissues. The above results should be useful in designing the most efficient stimulators for such use.

The above calculations were originally made because of their application to nerve stimulation from an external source. It has been pointed out by one of the editors of this journal that the results are also applicable to the stimulation of nerve by its own action currents: *i.e.*, to nervous conduction. Rashevsky (6) shows that, at least in his assumptions, the action current is an exponentially rising current as it excites the axon during the progress of the nerve impulse. After some rearrangement of Rashevsky's equations, it is found that this action current is

$$i = \text{const. } e^{[(I-R)/R] kt}$$

so that if the peak action current  $I$  is twice the rheobasic value,  $R$ , the waveform is the optimum, and the action is propagated with minimum energy.

Blair (1) found the ratio  $I/R$  to be approximately 2 in normal nerve, so on the above basis, action is actually propagated with minimum energy.

#### SUMMARY

An exponentially rising current will stimulate nerve with least power. Such waves are not easily generated. A square wave of correct intensity only requires 22 per cent more power, and is easily generated. The exponentially falling current obtained from thyatron stimulators requires 85 per cent more power than the best current form. The use of square waves for stimulators and electric shock therapy is indicated.

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# RESPONSES OF SINGLE HUMAN MOTOR UNITS TO ELECTRICAL STIMULATION

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## INTRODUCTION

CLINICAL electromyography has usually been limited to studies of voluntary contractions or of spontaneous activity in the muscle but a systematic analysis of muscular function using electrical stimulation of the nerve, such as is common in animal experiments, has been less used in clinical investigations. In previously published experiments of this kind synchronized potentials from many simultaneously active units resulting from maximal or sub-maximal test shocks of short duration have been recorded (2, 3, 5, 7). These mass effects are difficult to interpret even under physiological conditions and are therefore far from ideal for the analysis of pathological phenomena. It is, therefore, desirable to find a method which would enable one to study the responses of individual human motor units to electrical stimulation.

This paper describes a modification for human material of Skoglund's (11) method for recording the activity of individual motor units to electrical stimulation with currents of different gradients. Although the object of the method was originally clinical, the application has for the present been limited to the study of some physiological problems which emerged from Kugelberg's study of accommodation in human nerves (9). Therefore a determination was made of the accommodation of a single motor unit and it is a matter of interest to compare these results with earlier data from experiments in which the muscle twitch was used as an index. The technique has also been used to detect differences in excitability between the proximal and distal parts of the same nerve fibre.

## TECHNIQUE AND PROCEDURE

The stimulators, which deliver exponentially or linearly rising currents, have already been described in detail (11). An important characteristic of the design is the high internal resistance of the output valve (150,000 ohms) whereby the form and strength of the stimulating currents are relatively little affected by changes in the resistance of the skin. Monopolar stimulation was employed with the cathode as the active electrode over the nerve. The cathode consists of a thin plate of silver (1.5 cm. in diameter) at the bottom of a thin round ebonite cup filled with asbestos. A thin silver plate of about 10 sq. cm. covered with chamois leather was used as the indifferent electrode and this was placed either on the upper arm or on the calf. Both electrodes were moistened with a 4 per cent sodium chloride solution and were fixed in position with strips of adhesive tape. The strength of the stimulating current was measured with an ammeter placed in series with the stimulating electrodes. The form of the current was recorded on one beam of the cathode ray tube, which was also used for the time marker. Concentric needle electrodes were used for leading off the action potentials to a condenser-coupled amplifier connected with the other beam of the tube. The subject was earthed with an extra electrode applied near the recording electrodes, an arrangement which proved effective for reducing stimulus artifacts.

All records in this paper are from the first dorsal interosseous muscle, since this was found to be especially suitable for artificial stimulation via the ulnar nerve at elbow level. However, successful recordings were also made from other muscles.

## RESULTS

### 1. DEMONSTRATION OF SINGLE UNIT RESPONSES

a. *Instantaneously rising currents.* Animal experiments suggest that certain difficulties will arise in any attempt to obtain responses from individual motor units when the stimuli used are such as to produce simultaneous discharges from many units (cf. 4, 11). And it was indeed found that systematic

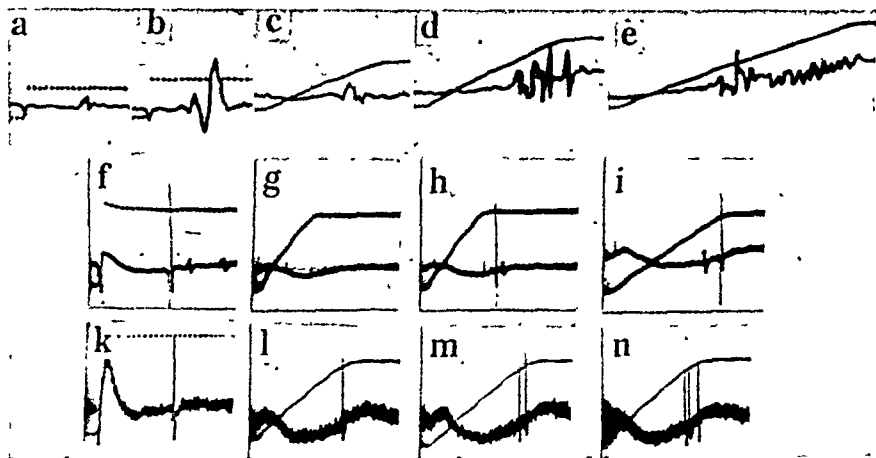


FIG. 1. Recording from the 1st dorsal interosseous muscle. Stimulation of the ulnar nerve at elbow level. Action currents on one beam; stimulus form and time marking on the second beam. Time intervals 2 msec. (interruption of the beam) and 20 msec. (superimposed sinus waves). Rising times and stimulus strengths are: a: rheobase, 1.2 mA; b: rheobase, 1.7 mA; c: 100 msec., 1.85 mA; d: 120 msec., 2.2 mA; e: 160 msec., 3.15 mA; f: rheobase, 0.80 mA; g: 45 msec., 0.80 mA; h: 50 msec., 0.90 mA; i: 120 msec., 1.45 mA; k: rheobase, 1.6 mA; l: 80 msec., 2.0 mA; m: 88 msec., 2.2 mA; n: 88 msec., 2.2 mA.

trials of different needle positions in the muscle had to be made before satisfactory results could be obtained.

In Figure 1, a-e show some, but not satisfactory, isolation of the action potentials. These records are published as a contrast to the perfect isolation attained from a single unit in Figure 1f-n as well as in Figure 2b-c. Let us first compare the responses to instantaneously rising currents. In Figure 1 we see how an increase of stimulus strength from 1.2 to 1.7 mA alters the record from the barely visible response of 1a to the large potential of 1b. The effect when isolation is perfect shows a sharp contrast. In Figure 2a the stimulus strength is below threshold for the unit in question (the movement of the base line is an artifact due to the application of the stimulus, but although the distortion is unusually large it is of too short a duration to interfere with the recording of the physiological potentials), while in Figure 2b, where the stimulus strength is just at the threshold, a potential of

short duration suddenly appears. This all or none reaction at the threshold is the chief sign that one "unit" only is involved. (We do not know whether one isolated spike in this case represents a response of a single motor unit in the strictly anatomical sense; for further discussion of this point see 11.) In addition, this spike potential shows the characteristic repetitive response to a stimulus of supra-threshold strength while remaining unchanged in size and form (record c). This result is in agreement with those obtained from animal experiments under the same conditions (4, 11).

b. *Slowly rising currents.* Accommodation, *i.e.*, the process in the nerve opposed to excitation which causes a rise in threshold in inverse relation to the rate of rise of the stimulus, occurs in these experiments (6, 12). Clearly, this phenomenon finds different expressions depending on whether the index is a composite action potential or a single unit response. Animal experiments have shown that it is in the latter case that excitation and its counterprocess can be best demonstrated.

In the uppermost series of records in Figure 1, those illustrating poor isolation, c shows how, owing to its slow rise, a stimulus of 1.85 mA has a weaker effect than that of an instantaneously rising stimulus of lower strength (1.7 mA) in b. The action potential in c is of smaller amplitude than in b, indicating the recruitment of fewer elements. In d the gradient is the same as in c, but the current reaches a higher final value (2.2 mA), so that the accommodation is to some extent counteracted and the result is a potential of greater amplitude. The action potential also lasts longer, due both to recruitment of new elements with higher thresholds and to repetitive responses from the units first activated (*cf.* 11). This continuous discharge during the rising phase of the stimulus is typical and is still more marked when the gradient is not so steep (see record e).

The results obtained under otherwise identical conditions but with perfect isolation are shown in Figure 1f-i. Record f shows the response of the unit in question to the rheobase current (0.8 mA). The effect of accommodation in raising the threshold, when more slowly rising currents are used, is clearly shown in g where the final current strength is unchanged. Since the stimulus is now of sub-threshold strength there is no response at all. With the same gradient an increase of current strength (to 0.9 mA in h) is necessary for the excitation of this unit. Record i shows that when the current rises still more slowly a further rise in final strength is necessary in order to evoke a response. For obvious reasons, the latency of the discharge is the more lengthened the more slowly the current rises.

The characteristic responses of one muscle unit when the slowly rising currents go above threshold strength can be seen in Figure 1k-n. k shows the

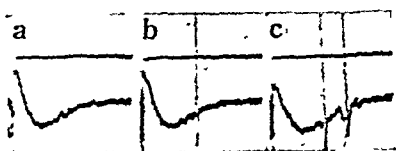


FIG. 2. All or none responses of a single unit to instantaneously rising currents (see text). Stimulus strengths are: a: 0.9 mA; b: 1.1 mA; c: 1.4 mA.

response of the unit to a rheobase stimulus of 1.6 mA and 1 the discharge when the current rises more slowly to a final strength of 2 mA. In m and n the gradient is unchanged, but the final current strength is increased to a supra-threshold value of 2.2 mA and we see how, during the rising phase, there is a repetitive discharge of two or three spike potentials depending on the level of excitability. This phenomenon is identical with that observed during animal experiments.

Stimulation with linearly increasing currents such as those used in the experiments described above has obviously advantages if one wishes to correlate the form and strength of the current with the characteristics of the discharge (cf. 11). In clinical practice, however, exponentially rising currents are usually employed since they are easier to produce. It can be seen

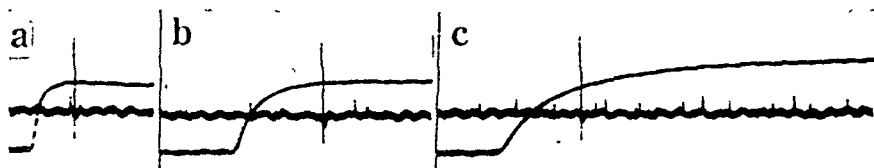


FIG. 3. Responses of a single unit to exponentially rising currents. (The small spikes on the base line are artifacts due to the camera motor.) Time constants and final current strengths are: a: 10 msec., 0.8 mA; b: 30 msec., 0.9 mA; c: 100 msec., 1.5 mA.

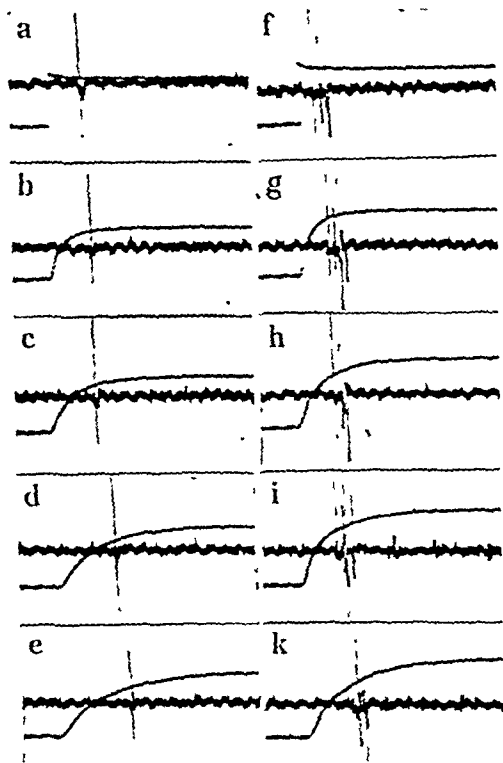
from Figure 3—in which the threshold response of a given unit to various exponential currents of different time constants are shown—that the results of the two methods are similar.

2. DETERMINATION OF ACCOMMODATION CURVES WITH A SINGLE UNIT INDEX. Kugelberg, using exponential currents and minimal muscle twitch as index, has made a thorough study of accommodation in human nerves, normal values and their variations being determined for most of the large muscle nerves (9). But, as Kugelberg points out, the muscle twitch, since it represents a large number of units, cannot be considered satisfactory as an index from a strictly physiological point of view. It should therefore be of interest to make a direct experimental comparison between the accommodation curve obtained by the classical method with muscle twitches and that determined with a single unit response. For this purpose such a comparative investigation was carried out in the following way. The needle electrode having been satisfactorily placed, first the rheobase value and then the successively rising thresholds for the exponential currents were determined, using the action potential of the isolated unit, as observed on the cathode ray, as an index (see Fig. 4a-e). The rheobase value and the threshold values of the exponential currents were then again determined, using the least muscle twitch as the index. The needle was left untouched to register the action potentials corresponding to these minimum twitches, and it is

quite obvious from the records (Fig. 4f-k) that the twitch is accompanied by discharges from several different units.

The same result appears from the accommodation curves in Figure 5. The threshold values, reduced to multiples of the rheobase, are plotted

FIG. 4. Responses to exponential currents with different time constants (fully described in text). a-e responses from a single unit of low threshold; f-k action currents corresponding to the least muscle twitch visible. Time constants and strengths of the stimulating currents are: a: rheobase, 0.7 mA; b: 20 msec., 0.8 mA; c: 40 msec., 1.0 mA; d: 75 msec., 1.2 mA; e: 100 msec., 1.5 mA; f: rheobase, 0.9 mA; g: 20 msec., 1.3 mA; h: 40 msec., 1.45 mA; i: 75 msec., 2.0 mA; k: 100 msec., 2.5 mA.



against the time constants of the exponential currents. The upper accommodation curve was obtained when the minimum muscle twitch was used as

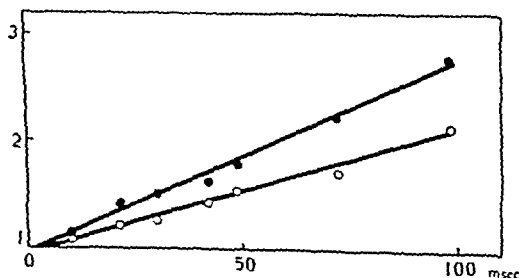


FIG. 5. Accommodation curves determined with a single motor unit as index (open circles) and with a minimum muscle twitch as index (filled circles). The slopes are 11.5 and 17.7 respectively (see text). Abscissae: time constants of the exponential currents in msec. Ordinates: threshold values in multiples of the rheobase value.

an index, and its slope (17.7) tallies well with Kugelberg's results. The lower accommodation curve obtained with the response of a single unit as index has a slope of only 11.5.

This difference between the two accommodation curves can be explained in several ways. First, it may be due to difficulty in determining the threshold when a muscle twitch is used since a quick contraction, caused by the simultaneous activity of a number of elements, is easier to observe than a slow one, caused by the successive response of different elements to a slowly rising current. Secondly, it is possible that there is an accommodation difference between the various elements (1, 11), those of low threshold exhibiting less accommodation than the high threshold ones, which are active when a visible muscle twitch is used as index.

In animals, a more satisfactory method of determining accommodation

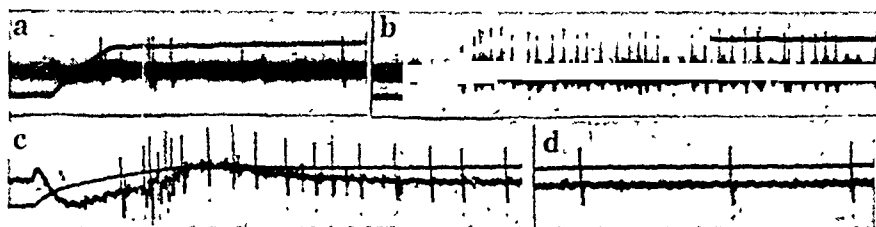


FIG. 6. Responses to constant currents of different strength and duration (fully described in text).

is that in which the threshold strength of currents of different gradients is calculated on the basis of the latency of the response (11). This method is, however, not suitable for human experiments, owing to great variations of the latency at the threshold. Under these conditions an increase in latency of about 10–15 msec. over the value for maximum stimulation has been observed.

3. RESPONSE TO CONSTANT CURRENTS. THE PROCESS OF ADAPTATION. It has long been known (see 9, 10) that human sensory and motor nerves both respond to constant currents with a continuous discharge. The technique described here allows an exact study of both the frequency and the duration of single unit discharges to be made. Figure 6a shows the responses to a current rising linearly to a final strength of twice the rheobase. It will be seen that a few repetitive discharges of irregular rhythm appear during the plateau phase in addition to the ordinary response while the current is still rising. In b an increased current strength—up to three times the rheobase—evokes a similar discharge, more regular, of higher frequency and of longer duration. The characteristic diminution in frequency cannot be seen in this record but is clearly shown in c and d taken from another experiment in which a current strength of 3.5 times the rheobase was used. In this experiment an initial frequency of 15 per sec. (c) fell to 6 per sec. after 6 seconds stimulation (d) and 4 seconds later the unit had stopped discharging altogether (after a total “adaptation time” of 10 sec.). This behaviour of the individual human motor unit during the stimulation of the intact nerve is similar to that found in animal experiments.

It has been found that the adaptation of a nerve to a constant stimulus is related to its accommodation, nerves with less accommodation exhibiting longer adaptation times (4, 8). In studies of the excitability of human nerves under certain pathological conditions—experimental ischaemia and tetany—Kugelberg found a reduced accommodation which was especially marked in the proximal part of the long nerve fibres of the arm (9). He also suggested that differences in excitability between the proximal and distal parts of the nerve fibres under normal conditions may exist, although no certain differences in accommodation could be demonstrated with the technique

Table 1

Stimulus strength (multiples of rheobase)	Case No.													
	1		2		3		4		5		6		7	
2	15	0	25	0										
3			60	30	6	2	0	0	0	0	1	0	2	0
4					7	3	4	0	10	0	30	15	15	5
5					15	10	13	0 5						

The first and second figures in each case show the total adaptation times in seconds at proximal and distal stimulation respectively. See text.

employed. It should be of interest, therefore, to investigate this point by using the total adaptation time as an index. For this purpose two active electrodes were placed over the ulnar nerve, one near the axilla and the other at wrist level. The responses were observed in the first dorsal interosseous muscle. The rheobase value for each electrode position having been determined for the same motor unit, constant currents of a strength equal to a selection of rheobase multiples were applied and the total adaptation times determined. The results are assembled in Table 1. In case 1 a constant current of twice the rheobase strength evoked a discharge lasting 15 seconds when applied to the proximal electrode but gave no repetitive firing at all if it was applied at the wrist. In case 2 the corresponding values were 25 and 0 seconds, while a constant current of three times the rheobase elicited a discharge that lasted one minute in the case of proximal and 30 seconds in the case of distal stimulation. With currents of higher strength the adaptation times for the low threshold units were difficult to determine owing to the infinitely slow adaptation, and in these cases it was found more suitable to determine the adaptation time by observing the duration of the muscle contraction. By this method the differences in adaptation times were quite obvious (see Table 1, cases 3-7).

Thus, there are definite differences in excitability between the proximal and distal parts of one and the same fibre of a human nerve. There is no reason to assume that these are due to the physical properties of the tissues surrounding the nerve when one uses a stimulator with the characteristics described above. That such a difference in adaptation—and consequently also



in accommodation—does exist under normal conditions is, of course, of great physiological interest and invites one to further experimentation on animals. These differences in excitability may bear some relation to the diminution in diameter of a fibre caused by peripheral branching. In the present work our only purpose is to draw attention to the pathophysiological significance of our result. It explains in a simple way why the clinical symptoms in ischaemia and tetany appear first and are more pronounced in the proximal parts of the nerve when it is exposed to influences causing decreased accommodation (cf. 9).

### SUMMARY

1. The responses of single human motor units to stimulation with instantaneously or slowly rising currents are demonstrated.

2. Accommodation curves with the electrical response of a single motor unit as index were determined and are compared to those given by a muscle twitch.

3. The duration of the motor unit responses to constant currents of various strength—the so-called adaptation time—was determined. Significant differences were found to exist for the proximal and distal parts of the same fibre, the former showing a longer adaptation time.

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# NATURAL AND ARTIFICIAL ACTIVATION OF MOTOR UNITS—A COMPARISON

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## INTRODUCTION

IN PREVIOUS investigations (5, 11, 16, 20, 21) the responses of nerve fibres to slowly rising currents were analyzed by means of a special technique. Such experiments, in which the course of the stimulating current and the nervous discharge were recorded simultaneously, can also be arranged to imitate certain mechanisms in the sense organs and spinal cord, in both of which slow potentials serving as exciting agents have been demonstrated (2, 3, 4). It has been shown that the discharges from sense organs are of the same character as the autorhythmic responses of sensory nerve fibres stimulated with slowly rising currents (e.g., 5, 11).

Since the responses of single motor units to electrical stimulation can now be studied in man (16), it should be possible to establish a direct comparison between such artificially evoked responses and the natural discharges of single motoneurons in voluntary contractions. It was shown by one of us (20) that the spike pattern in cat muscles as a result of stimulation of the nerves with slowly rising currents is characterized by an initial appearance of small spikes followed later by larger ones corresponding to units of higher threshold. In this way units of different thresholds can be studied separately.

In the experiments described in this paper, the typical "recruitment" of different units to electrical stimulation already found in animals has been verified in man. If these results are compared with those obtained during voluntary contractions, striking similarities are revealed. Thus, it was found that the sequence of activation of different units observed during voluntary contraction was very like that occurring during electrical stimulation and that the frequency relations between the discharges of the various units activated by both types of contraction were also nearly identical.

## TECHNIQUE

The stimulator, which is designed to deliver linearly rising and constant currents of strictly controllable gradient and strength, has already been described in detail (20). It was designed to work automatically when relatively steep current gradients were required and the form of the current was recorded on one beam of the cathode ray tube (see Fig. 1). For very slow increase of current the adjustment was made by hand and in these cases the shape of the current was not recorded. When the current strength was measured this was done by means of an ammeter in series with the stimulating electrodes. Monopolar stimulation was used with the cathode as active electrode placed over the nerve. (For further details of stimulating electrodes see 16.) Concentric needle electrodes were used for recording the action potentials to a condenser-coupled amplifier connected with the second beam of the cathode ray tube. The other beam was used for time recording, except in some experiments in which double leads were employed. The mechanical muscle effects were not regis-

tered. Experiments were carried out on four different subjects. Two muscles, the first dorsal interosseous muscle (*stimulation of the ulnar nerve at the elbow*) and the peroneus longus muscle (*stimulation of the peroneal nerve at knee level*), were investigated.

## RESULTS

1. GENERAL DESCRIPTION OF TYPICAL ACTIVATION OF DIFFERENT UNITS DURING ELECTRICAL STIMULATION. In order to study the relationship between the activities of different units it is important that the needle position is such that the responses of more than one unit (cf. 16) can be recorded. Figure 1 illustrates the reaction of three separate units of different threshold.

Record a shows the response of the lowest-threshold unit (A) to an instantaneously rising current. In this case the threshold of the unit was reached at 0.65 mA, but when a current rising with the gradient shown in b is used the threshold strength rose to 0.8 mA; this is of course due to accommodation (cf. 20). In record c a current of the same gradient but reaching a higher final value (0.85 mA) produces a second response of greater amplitude from a new unit (B) (100  $\mu$ V as compared with 50  $\mu$ V for unit A). The same result—lower threshold of A than of B—is demonstrated with another and slower current gradient in d and e. Records f to n were taken on a compressed time scale (see Fig. legend). In the experiment recorded in f a still more slowly rising current produced a single response from A at a strength of 1.45 mA, while a supra-threshold stimulus (1.7 mA) by a current of the same gradient elicited a rapid repetitive firing from unit A during the rising phase (record g). This was followed during the constant phase by a discharge of low frequency. It has been shown previously that such a response is typical when a constant current reaches more than 2.5 times the rheobasic value (16, 20).

In g the current strength is strong enough to evoke a repetitive response from B during the rising phase but not during the plateau phase. To release the autorhythmic mechanism of this unit during the plateau phase the current had to reach a strength of 2.0 mA (record h). In this case the response of B is slow and irregular (cf. the behaviour of A in record g), while that of A is now more regular, of higher frequency and longer duration.

If the stimulating current is allowed to rise still further to 2.5 mA (record i) yet a third unit (C), giving a response of still greater amplitude (about 200  $\mu$ V), is activated. This unit starts its rhythmical firing during the plateau phase at a current strength of 3 mA (record l). (The frequency values of these units are assembled in Table 1.)

The current used in the experiment recorded in h elicited a discharge of higher initial frequency and of longer duration from A than from B. This was to be expected, since it has been shown in experiments on single neurons (11, 20) that both the frequency and the adaptation time are functions of the strength of the current in relation to the rheobase value. (The rheobase for B and C could not be determined directly in this experiment, since the effects of many units will be synchronized when the stimulating current rises instantaneously, but the approximate value can be calculated from the threshold obtained with the steeper gradients.) It is obvious that, in terms

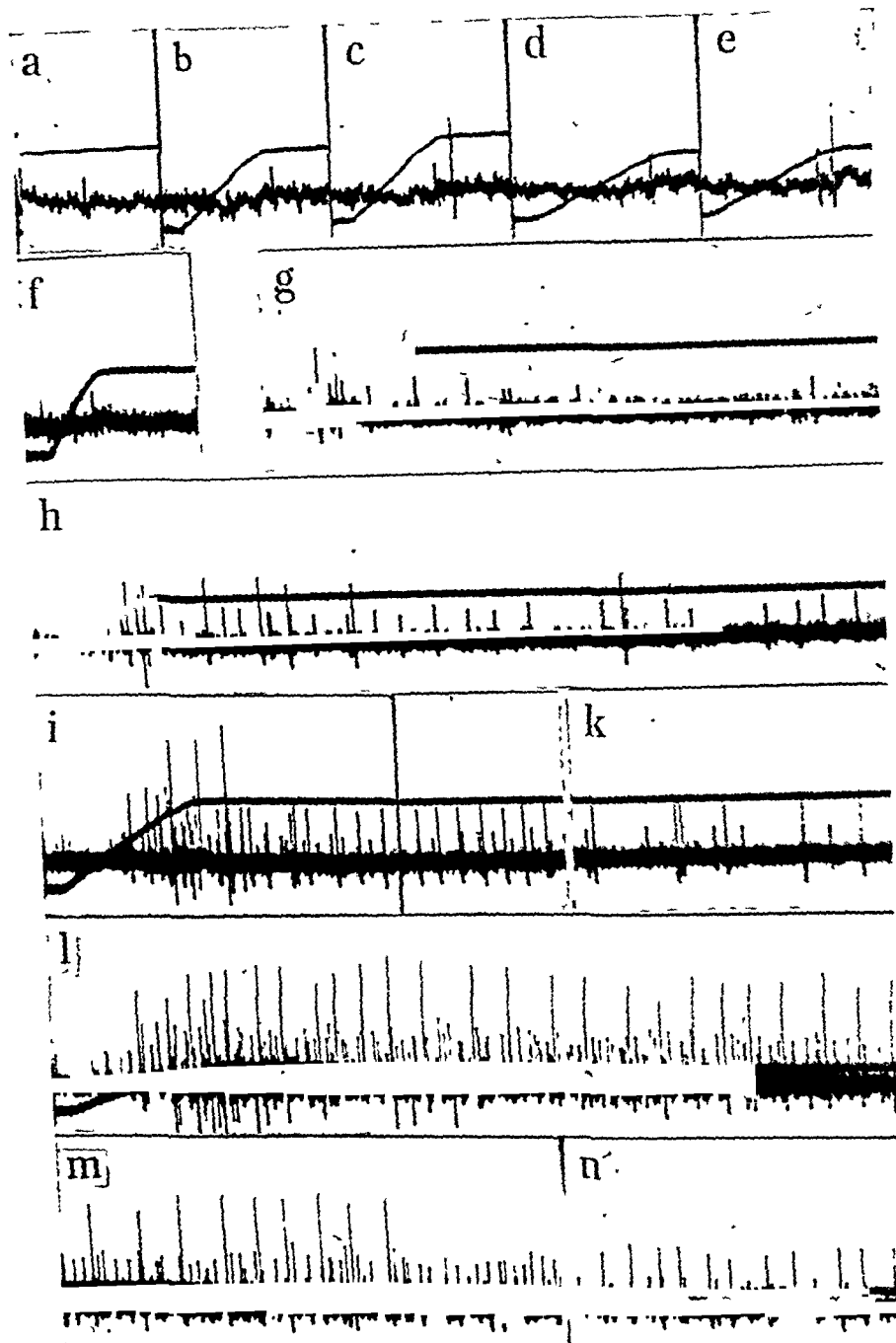


FIG. 1. Responses from three different units in the first dorsal interosseus muscle. Stimulation of the ulnar nerve with linearly rising and constant currents. Stimulus form and time marking on the same beam. The time intervals are in a-e 2 msec. and in f-n 20 msec. (There is no proportionality between the strength of the stimulus and the deflexion of the beam recording stimulus form in the different records.) (Fully described in text.)

of their respective rheobasic strengths, the current in h is stronger for A than for B, the values being 3.2 and 2.65 times the rheobase respectively. The latter figure is about the same as that for A in g (2.6 times the rheobase) and it will be seen that the responses (*i.e.*, an irregular discharge of 4-5 impulses) of the two units are also very similar.

As one might expect, the higher absolute current strength used for record i evoked discharges of higher frequencies from both units, the initial fre-

Table 1

Experiment	Unit	Frequency			
7 (Fig. 1) Electrical stimulation	A	6-7	10	25	*
	B	—	5-7	12	50
	C	—	—	irregular <5	7
11 (Fig. 7) Voluntary innervation	A	5-10	25	40	*
	B	—	irregular <5	15	25
	C	—	—	—	irregular <5
Electrical stimulation	A	5-10	30	50	*
	B	—	irregular <5	20	25
	C	—	—	—	irregular <5
16 Voluntary innervation	A	10	15-20		
	B	—	5		
Electrical stimulation	A	5	15		
	B	—	5		

\* Could not be determined.

quencies being 25 and 12 per sec. respectively. After 3 sec. the frequencies fell to 8 and 5 per sec. (record k) and a little later B dropped out altogether. Record m, a direct continuation of l, illustrates similar behaviour on the part of C. At this point the frequencies of A and B have decreased from a value which was too high to count (in l) to 25 and 18 per sec. respectively; after another 6 sec. (record n) these have further decreased to 16 and 10 per sec. The unit of lowest threshold is always the last to disappear.

This behaviour follows from the relation between adaptation time and relative current strength already mentioned. However, another factor may also be of importance in this connection. If fibres of different size exhibit the differences in accommodation suggested by animal experiments (20), these would influence their adaptation times in the same way as do their threshold differences (*cf.* 11, 13). Experiments on man do not lend themselves to a study of this problem. These differences in adaptation to a constant current are of small interest in the investigations to be described; they have merely been touched upon in order to complete a general description of the re-

sponses of different units to electrical stimulation. The important point is that different units can be studied separately if stimulation with slowly rising currents is employed.

It is obvious that discrimination between units of different threshold will be easier the more slowly the current rises, but when the gradients are very low currents lose their capacity to stimulate during the rising phase. In this case a unit starts firing rhythmically as a result of stimulation by the current in its plateau phase, the frequency of the discharge being determined by the relative strength of the current, independently of the rate at which this has been reached (cf. 11, 20). The thresholds of different units in an experiment of this type remain constant and there are, as a rule, very marked differences between them. Units always differ markedly in the amplitude of their response and this also makes differentiation easier. The relation between the anatomical size of a unit and its spike size will be discussed later.

2. COMPARISON BETWEEN VOLUNTARY CONTRACTION AND ELECTRICAL STIMULATION. When a nerve is stimulated by a very slowly rising current (see section on Technique) the mechanical effect in the muscle is a tetanic contraction very similar to a slow voluntary movement and we will now compare the spike patterns resulting from these two sustained contractions of different origin. Brief muscle twitches, whether voluntary or evoked by steeply rising currents, are not dealt with in this paper.

Figures 3-7 show that there are striking similarities between the spike patterns characteristic of the two types of contraction. These records will be systematically analyzed from the point of view of (a) the order in which different units are activated, (b) their identification and (c) their discharge frequencies.

a. *Order of activation of units of different spike sizes.* The most obvious similarity between the two types of contraction is that units giving small amplitude potentials are the first to be activated and that these are followed by ones with larger spikes (see Figs. 3-7). This behaviour has already been observed for voluntary contraction but has been differently interpreted by different investigators. Smith (23) pointed out that the amplitude of the response is a function, not only of the potential changes in the muscle fibres, but also of their distance from the electrodes. He concluded that "since the number of units at a given distance increases with the square of the distance from the active electrode, it may be merely on these grounds that distant activity is the first to appear and the last to disappear." Denny-Brown and Pennybacker (7), however, assumed that there is a direct relationship between the spike size recorded and the size of the motor unit. They suggested that the earlier activation of small units serves to smooth the contraction. Eccles and Sherrington (8) proved that the motor units vary in size, but whether the small or the large units have the higher threshold when reflexly excited has never been determined. The anatomical basis of different spike patterns cannot be determined in the present investigations but our experi-

ments with electrical stimulation seem to support the view that the order of activation of units of increasing spike sizes is, in fact, the expression of a functional organization of the muscle.

Our results with electrical stimulation (cf. section 1) certainly suggest that the small potentials initially recorded from the muscle must be due to activity in the largest fibres of the motor nerve since these have the lowest threshold. If one accepts a direct relation between the anatomical size of a motor unit and its spike height one must also accept, contrary to the general assumption (8), that small units are innervated by large nerve fibres. It is, however, idle to indulge in further discussion of this point with our present

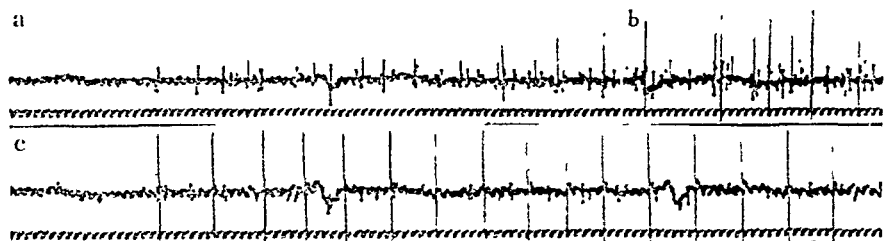


FIG. 2. Records from the first dorsal interosseous muscle. a and b, normal voluntary contraction. c, voluntary contraction during the initial stage of ischemic paralysis. (The slow diphasic potentials are due to heart activity.) In this and the following figures the time intervals are 20 msec.

scanty knowledge of the electrophysiology of muscle, since we have no evidence that the single spike recorded by a needle electrode in the muscle always corresponds to a motor unit in the classical sense, *i.e.*, a group of muscle fibres innervated by a single anterior horn cell. Experience with animal experiments (20, 22), as well as with pathological human material (6, 15), indicates that synchronized action potentials, representing the activity of larger aggregates of fibres, may appear but may nevertheless exhibit an "all or none" reaction within certain limits. It is not possible, with our present knowledge, to come to any definite conclusion as to the relationship between spike height and unit size. Nor is it relevant to the present work, since we only use the differences in amplitude as a means of differentiation between several simultaneously active units.

As will be shown later, the spike patterns evoked by voluntary contraction and electrical stimulation are very similar. This fact suggests that in the first case, as in the second, the initial small spikes are produced by impulses in the same low threshold nerve fibres. That this is probably the case can be shown more directly by selective blocking of the fibres in the nerve stem by means of ischemia. This condition affects the coarse fibres first (*e.g.*, 17) and should, therefore, cause the small spikes, characteristic of the first stage of a voluntary contraction, to disappear. This was, in fact, the case in experiments carried out on two different subjects. The effect on the action potentials recorded in the muscle when the arterial circulation to the nerve is cut off by means of a cuff around the upper arm is shown in Figure 2. Records a and b show the usual order of activation of units giving spikes of successively increasing amplitude during the initial (a) and later stages (b) of a contraction under normal conditions. Record c was taken during the first stage of partial paralysis resulting from 20 minutes' arrest of the circulation. The smaller spikes are now absent and the largest are the first and only ones to appear. These are of about the same

size as those recorded in *b* during a strong normal contraction. One should, however, exercise some reserve in assuming the two spikes shown in *b* and *c* to be identical, since the abnormal condition in muscle and nerve during ischemia may give rise to abnormal synchronizations. In any case, no small spikes appeared until after the pressure had been released when, as a result of spontaneous activity in the nerve (14, 17), repetitive firing from units of different size was observed. Some minutes later, after this discharge had ceased, a voluntary contraction gave the same spike pattern as that recorded before the ischemia.

*b. Identity of units.* It is common knowledge that a voluntary movement always starts with the activity of a particular motor unit (e.g., 10) and we

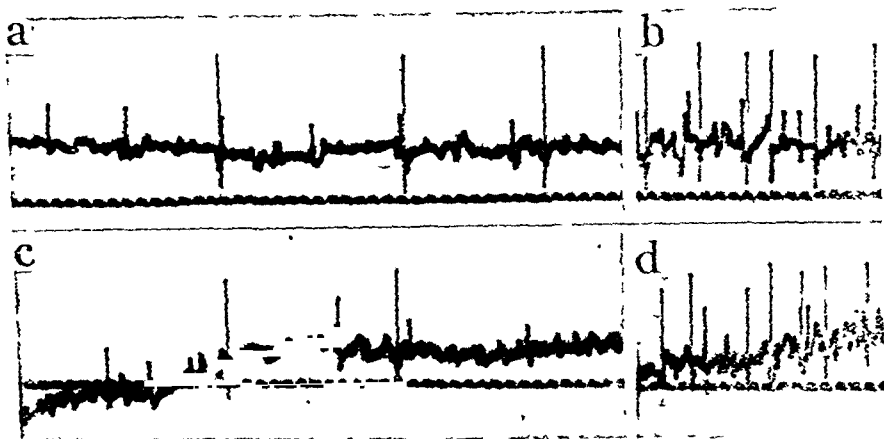


FIG. 3. Records from peroneus longus. *a* and *b*, voluntary contraction. *c* and *d*, electrical stimulation. (Further description in text.)

found that this is also the case when electrical stimulation is used. The thresholds of the subsequent spikes also remain constant throughout an experiment. The question now arises as to whether the units activated during a voluntary contraction are identical with those of similar character recruited by electrical stimulation.

In Figure 3 the spike pattern during voluntary contraction is shown in *a* and *b* and should be compared with that due to electrical stimulation given in *c* and *d*. The voluntary contraction starts with a small discharge of about  $50 \mu\text{V}$ , followed by a larger one of about  $150 \mu\text{V}$ . The spike potentials elicited by the slowly increasing currents used in record *c* are apparently similar to those in *a* as regards electrical sign, size and form, and this similarity is further confirmed by observations on the screen at high sweep speeds. From the identity of the electrical phenomena it may be concluded that, in this experiment, both the voluntary and the artificially evoked contraction started with activity of the same units.

In this particular experiment the pattern of the voluntary contraction is characterized by the small number of initial spikes of low amplitude which precedes the appearance of the larger spikes associated with the second unit to become active; this was the case however slowly the contraction was increased. The response to electrical stimulation indicated the same difference between the thresholds of these two units.



Further evidence that the same units can be activated by both voluntary contraction and electrical stimulation is presented in Figure 4. In this experiment two electrodes separated by about 3 cm. were inserted into the peroneus longus. In Figure 4 the activity resulting from different strengths of voluntary contraction are recorded in a and b, while the records taken during electrical stimulation of different intensities are shown in c and d. Let us first compare the activities registered by electrode II (on the lower beam). It will be seen that the three different spikes occurring in record a (a very small one of about  $50\mu\text{V}$ , a medium one of  $100\mu\text{V}$  and a large one of about

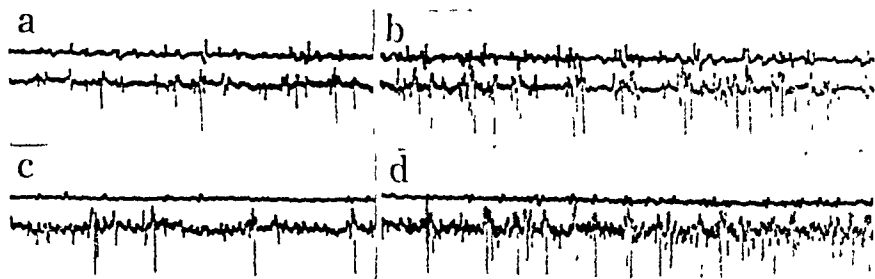


FIG. 4. Double leads from peroneus longus. a and b, voluntary contraction. c and d, electrical stimulation. (See text.)

$200\mu\text{V}$ ) also appear in record c. During the more intense voluntary contraction (record b) the pattern is characterized by groups of spikes due to repetitive discharges from these three units. This type of discharge is exactly reproduced when the nerve is stimulated electrically (record d). In fact it would be impossible from their spike patterns to distinguish between the two types of contraction. (The somewhat broader base line in records c and d does not represent increased muscular activity but is due to electrical disturbances picked up by the amplifier when the subject is connected to the stimulator.)

During both types of contraction, electrode I (on the upper beam) picked up very little activity as compared with electrode II, but a comparison of the activities which were recorded in the two cases reveals some discrepancies. Two units (which discharge independently of those recorded by electrode II) are certainly activated by voluntary contraction (a and b) but only one, which may or may not be identical with the smaller of these, by electrical stimulation (c and d). The relative threshold of the larger unit is evidently higher for electrical stimulation than when it is activated in voluntary movement. This is not the only case in which different parts of a muscle have been observed to produce differences in the spike pattern associated with the two types of contraction.

A contrary result—i.e., activation of a particular unit by electrical stimulation earlier than in voluntary contraction—is illustrated in Figure 5 in which the spike pattern of voluntary contraction is shown in records a and b and that obtained during electrical stimulation in c and d. These four

records exhibit the usual similarity. However, after repeated electrical stimulation (in e and f) a new medium-sized spike of about the same threshold as the small one in c and d suddenly appears. This spike was not present in the records of control experiments with voluntary contraction. It is clear that this unit's threshold to electrical stimulation must have changed during the experiment. There are, of course, many possible explanations of such variations in the relative thresholds of different fibres of a nerve stem, especially when these are stimulated through the skin.

As a rule, however, the thresholds of different units bear a very constant relation to one another, and this is also the case when the motor nerve is stimulated at different points. For instance, in Figure 6 the activity evoked on the first dorsal interosseus muscle by stimulation of the ulnar nerve at the wrist is shown in record b and that when the stimulus is applied at the elbow in record c. The two spike patterns are very much alike and of the same type as that produced by voluntary contraction (record a).

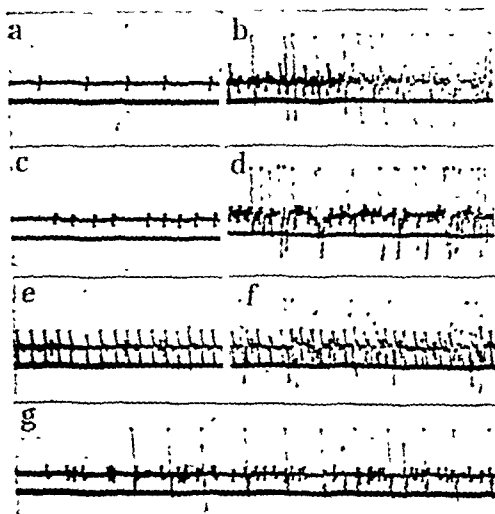


FIG. 5. Records from peroneus longus. a, b and g, voluntary contraction. c-f, electrical stimulation. (Fully described in text.)

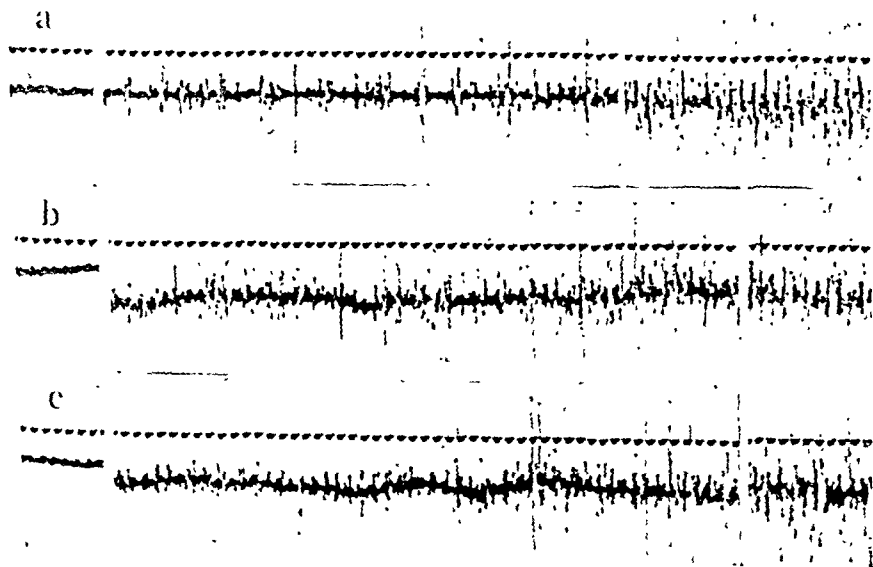


FIG. 6. Records from the first dorsal interosseus muscle. a, voluntary contraction. b and c, electrical stimulation of the ulnar nerve at wrist and elbow levels respectively.

Figure 7 provides a further illustration of the general rule that the same muscle units are activated both during voluntary innervation and electrical stimulation of the nerve. The spikes are of exactly the same size and shape in both experiments.

*c. Frequency of discharge.* Adrian and Bronk (1) were the first to study the frequency behaviour of single motor units during voluntary contraction and their work was followed by many other investigations on the same subject (*e.g.*, 10, 12, 18, 19, 23, 24, 25), the principal results of which may be summed up as follows: the initial frequencies of the motor units associated with slight degrees of voluntary contraction range for the most part between 5 and 10 per sec. (regular frequencies as low as 3 per sec. have occasionally been observed although the threshold responses are usually quite irregular); the frequency of the unit discharge increases with increasing contraction up to a maximum of about 30–50 per sec. in healthy muscles and 80–90 in parietic muscles; the initial frequency for each new unit is, as a rule, lower than for those already involved, but with further increase of the contraction the new unit soon attains the same maximum frequency.

These well-established findings with voluntary contraction agree remarkably well with those in the experiment on electrical stimulation described in section 1. The lowest regular frequency seen in this experiment was 7 per sec. and the highest about 50 per sec. This latter value is the same as that obtained in strong voluntary contractions. Finally, a striking feature when electrical stimulation is used is that the earlier units in the sequence of activation have always attained a higher frequency by the time the later ones appear.

A direct comparison between the frequencies during both types of contraction may be seen in Figure 3. Here the intervals between the small initial spikes vary between 100 and 140 msec. in the case of the voluntary contraction and between 80 and 140 in the case of electrical stimulation (records a and c). The intervals between the discharges of the large units are also about the same in both cases. Records b and d show the discharges of both units after the contraction was increased. The two are now discharging at the same frequency of 20 per sec.

In Figure 4 records a and c (lower beams) clearly demonstrate how, in both types of experiment, the smallest unit has already reached a relatively high frequency (about 10 per sec.) by the time the largest unit starts firing. The characteristic grouping of the discharges of the different units at higher frequencies (in records b and d) has already been emphasized.

The initial rhythms of identical units at threshold in both types of contraction were generally the same, but sometimes a very small voluntary contraction gave a slower frequency than could be obtained by employing the weakest possible electrical stimulus (see records a and c in Fig. 5). In Figure 5e the rhythmical responses of the medium-sized unit evoked by electrical stimulation were very regular and this was also the case in the similar experiments recorded in Figure 1. However, this marked regularity

is not especially characteristic of electrical stimulation. Both irregular and regular discharges may be found in the two types of contraction studied. Thus the spike pattern of the voluntary contraction in Figure 5g is characterized by an irregular discharge of the small unit, while the large one exhibits a very regular rhythm from the beginning.

An exact determination of the frequencies of active units at the time of the entry of new ones was possible in some experiments in which the regular rhythms of several units could be followed. The records in Figure 7 are from

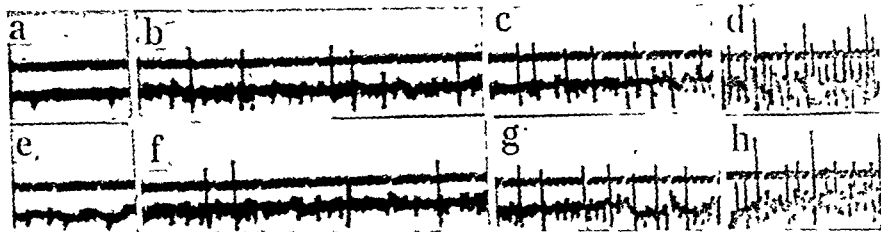


FIG. 7. Records from the first dorsal interosseous muscle. a-d, voluntary contraction. e-h, electrical stimulation. (Fully described in text.)

one of these experiments, the upper series being from a voluntary contraction steadily increasing in strength, the lower being recorded during electrical stimulation. In each the first unit to appear is of very small amplitude (about  $25 \mu\text{V}$ ) and can be recognized by the downward deflection of the base-line (see records a and e). The moment at which the next unit comes into action with a slow, somewhat irregular rhythm is shown in b and f. By this time the frequency of the first unit has reached a value of about 25-30 per sec. in both experiments. The next pair of records represents stages of still more increased contraction, in which the frequencies of both units have increased; the values being 40 per sec. for the first and 15 per sec. for the second in the case of the voluntary contraction, and 50 per sec. and 20 per sec. respectively for electrical stimulation. The last two records show the accession of a still larger spike at a time when the medium unit is discharging at a rate of about 25 per sec. in both cases. The frequency of the smallest unit has also increased, but its value cannot be determined because of interference from the large spikes.

The frequency values obtained from this experiment are assembled in Table 1, where some determinations from another comparative experiment will also be found, as well as the values from the one illustrated in Figure 1. The table shows both the initial frequencies of the different units and the frequency values attained by previously active units when new ones appear. It will be seen that there is a very close similarity between the discharge frequencies in both types of contraction.

#### DISCUSSION

In the case of electrical stimulation, the possibility of reflex responses caused by the simultaneous excitation of afferent fibres must be taken into

account. That proprioceptive fibres from the muscle have indeed been excited can sometimes be recognized by the subject by a feeling of tension, even when the stimulation is not strong enough to cause contraction. It is our experience, however, that rather strong synchronized centripetal volleys are necessary to evoke a reflex contraction, and such discharges are not produced by currents of the strengths used in these experiments. The possibility of involuntary contractions due to stimulation of pain fibres in the nerve (or of pain receptors in the skin under the active electrode) must be borne in mind, but this source of error can be excluded if the subjects are trained.

Blocking of the nerve proximally to the point of stimulation would be an effective method of excluding central effects, but we found it difficult to secure complete blocking of the whole nerve stem by pressure or cocaine, and further experiments were abandoned owing to the risks of much repetition of such treatment. On the other hand, the fact that the results were the same in man as they were in animal experiments, where of course the nerve was severed, may be considered a satisfactory indirect proof that we are dealing with peripheral effects only.

It must be emphasized that the experiments have been made only on the two muscles which proved especially suitable for artificial stimulation. Further, it was possible to study contractions of only submaximal strength, owing to the pain evoked by very strong stimulating currents. These two facts must naturally be borne in mind in considering the results.

The similarity of the responses from the individual motoneurons during voluntary contraction to those evoked by stimulation with constant currents supports Barron and Matthews' theory (2) that the natural discharges are caused by a depolarization of the intramedullary part of the neuron. The fact that the initial frequencies of the units are the same in both cases provides striking evidence in favour of the view that it is the inherent auto-rhythmic properties of the neuron which determine the frequency of the voluntary discharges.

The phenomenon of adaptation encountered in the case of artificial stimulation has, however, no counterpart during voluntary contraction, when each neuron maintains a constant rhythm for long periods (*e.g.*, 10). This dissimilarity might be explained by the existence of different properties in different parts of the neuron (the adaptation time to a current of given strength has been shown to increase towards the central part of the human motoneuron, *cf.* 16). In addition, it is quite obvious that the depolarization occurring in the spinal cord as a result of natural stimulation cannot be exactly imitated by constant currents applied externally.

One important new fact has emerged from these experiments. This is the parallelism between the thresholds of different motoneurons during electrical stimulation and central excitation. The detailed analysis of the type of experiment described in section 1 clearly indicates the part played by the relationship between the thresholds of different neurons in determining the discharges of their units. Further, the comparative experiments in section 2

show that this relationship is unchanged whether the contraction is voluntary or is initiated by a single potential applied to the motor nerve. It would, however, be an unjustifiable simplification to conclude from this that the central mechanism is represented by a single potential field depolarizing all neurons belonging to the same muscle. The very fact that the anterior horn cells which innervate a given muscle lie on different levels in the spinal cord makes such an explanation very unlikely. We must remember that the comparison only applies to the limited group of muscle fibres whose activity is recorded by a needle electrode in any given position. But, at least in the case of such limited groups, the similarity of the discharge frequencies of the successively active units in both types of experiment is so close that a similar excitation mechanism must be assumed.

The fundamental conclusion from our results is that the inherent properties of the different nerve fibres which determine the threshold differences to peripheral electrical stimulation surely have a significant rôle in the central excitation processes and play their own part within the functional organization of motor units. It is, therefore, all the more important to study the distribution and properties of the individual nerve fibre along the lines already laid down by Erlanger and Gasser (9).

#### SUMMARY

The activation of motor units evoked by voluntary innervation and by electrical stimulation of the motor nerve has been studied in certain human muscles.

1. Stimulation with slowly rising currents has made a separate study of motor units of different thresholds and spike sizes possible. A typical experiment, involving the response of three different units to linearly rising currents of different gradients and strengths and to constant currents of different strengths, is described.

2. The recruitment of motor units to nerve stimulation with very slowly rising currents has been shown to be similar in certain respects to that found during sustained voluntary contractions.

- a. Both types of contraction start with a unit of small amplitude, followed by units of progressively larger spike size. The electrical stimulation experiments, as well as some with selective blocking of the motor nerve during voluntary innervation, indicate that the initial small spikes from the muscle correspond to activity in low threshold nerve fibres. The factors determining muscle spike size are briefly discussed.

- b. In most experiments the units appearing in a particular order during a voluntary contraction are identical with those recruited in the same order by electrical stimulation.

- c. The initial discharge frequencies of identical units at threshold in both contractions are about the same. The increase of frequency during increased contraction exhibited by a given unit when the next in the sequence appears is also the same in both cases.

The results are discussed in the light of the theories of central excitation,

and special attention is directed to the parallelism between the thresholds of different motoneurons during peripheral stimulation and central excitation.

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# COMPETITIVE REINNERVATION OF RAT MUSCLES BY THEIR OWN AND FOREIGN NERVES\*

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LABORATORY experiments and surgical practice have amply demonstrated the fact that different motor nerves can substitute for each other; that is, a muscle deprived of its own motor nerve and connected with a foreign nerve will accept new functional innervation from the latter. This is invariably true when none but the foreign supply is available. The question remains, however, whether if the original and a foreign source are both made available to the muscle on equal terms, the original combination might not be favored. Elsberg (1), in a brief note, reports some experiments in rabbits that would seem to answer this question in the affirmative.

For brevity, let us designate the original nerve supply of a muscle as "O," and any foreign motor supply as "F." Elsberg, after confirming that both O and F can reinnervate a muscle if either is present without the other, claims that when fibers of both are allowed to interpenetrate the muscle concomitantly, functional connections will be established only by those coming from O to the exclusion of those coming from F. Such relative selectivity would be quite conceivable on general grounds. It has been definitely shown, for instance, that sensory nerve fibers cannot form transmissive junctions with muscle fibers (3, 10). This proves not only that sensory and motor fibers are different in character, but also that this difference is relevant to the establishment of functional connections. Since the principle of "myotypic" (or "homologous") function (5, 6) has made it axiomatic to assume that motor fibers, moreover, differ specifically among themselves in accordance with the different muscles on which they terminate, one could maintain that during the restoration of neuro-muscular connections concordant combinations would be favored over discordant ones.

The question thus becomes purely one of facts. Are the facts reported by Elsberg sufficiently conclusive to warrant the interpretation given to them? To judge from his description, they are not. The two nerves in competition were not given equal chances. The original nerve was cut and resutured, while the foreign nerve was inserted at random between the muscle fibers. The fibers of the former, therefore, had the whole pathway system of their own distal stumps available for easy permeation of the muscle, while the latter were forced to travel the hard way. The former would thus arrive at the scene ahead of the latter and occupy the muscle fibers, and since, as

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Elsberg himself found and as has been confirmed later (2, 11), a muscle fiber which is already innervated does not readily accept additional innervation, most of the foreign nerve fibers would remain unconnected—not by virtue of being different, but because of being late. The problem thus remained in need of reinvestigation under more crucial conditions. This was done in the following experiments.

#### MATERIALS AND METHODS

In these experiments, two nerves, one containing the old fibers (O) of a given muscle group, the other fibers (F) all foreign to that group, were placed in regenerative competition

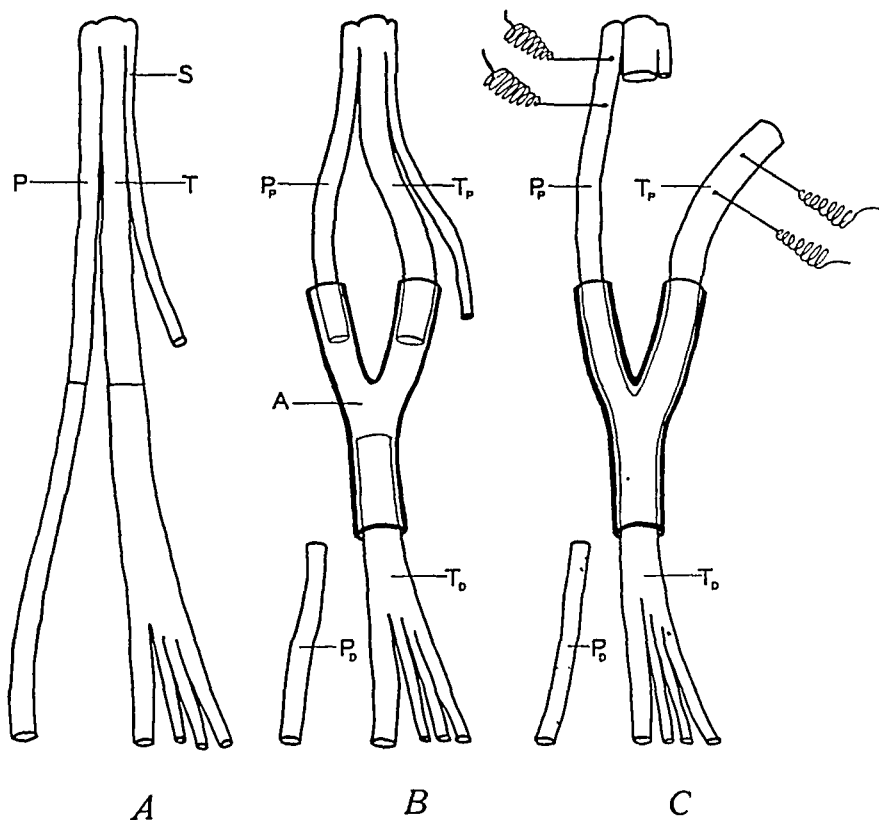


FIG. 1. Diagrams of operation (A, B) and terminal stimulation test (C).  
Explanation in text.

so that they had to use the same pathway and arrive jointly by the same route in the muscle. Conditions were thus as nearly alike for both as possible. Any subsequent superiority of O over F in reestablishing connections could then be ascribed to selectivity. It would have to express itself in a significantly larger quota of muscle fibers being taken over by O than by F.

The plantar extensor group (gastrocnemius, soleus, plantaris) of the white rat was used as test. All are normally innervated by branches of the nervus tibialis, none from the nervus peroneus. These two nerves were therefore chosen as O and F. The operation is explained in the diagram, Figure 1.

The tibial (T) and peroneal nerves (P) are cut in mid-thigh (Fig. 1A). The proximal stumps of both are then connected with the distal stump of the tibial only. This is done by the use of the Y-shaped lower end of the aorta (A) taken from another animal (Fig. 1B). This preparation, formerly used to divide a regenerating nerve in two (12), is here being used in reverse to make two nerves converge upon a single channel. The proximal nerve stumps (Tr, Pp) are inserted into the two iliac branches of the aorta, and the distal tibial stump (Td) into the trunk of the aorta. A blood-filled gap of about 2 mm. is left between the nerve ends so that none of them extend to the forking point of the sleeve. For the technique of the operation, see Weiss (7). The distal peroneal stump is partly evulsed and the rest (Pd) is left to degenerate. The sural nerve (S, Fig. 1) is left intact.

As has been explained in an earlier review (8), regenerating nerve fibers in arterial sleeves follow a longitudinal course across the gap. Thus, a mixture of both peroneal and tibial fibers arrive at the distal stump simultaneously. Since even sensory fibers are freely admitted into distal motor stumps even in the presence of competing motor fibers (10), it could be expected that both peroneal and tibial fibers would penetrate the distal tibial nerve indiscriminately. A major portion of this mixture would then be guided into the plantar extensor muscles.

The relative share taken by the tibial and peroneal fibers in the reinnervation of these muscles was determined by stimulation tests. The animals were biopsied from 49 to 129 days after the operation. The old wound bed was exposed and the proximal portions of the tibial and peroneal nerves were separated as high up into the common sciatic trunk as possible. The epineurium of the sciatic was slit open for the purpose. The nerves were then cut proximally, leaving free ample lengths (from 12 to 25 mm.) for stimulation without danger of current escape (Fig. 1C). The thigh of the animal was then tacked to a platform, and the Achilles tendon was attached to a steel wire connected with a torsion spring lever for isometric recording of tension. Vascularization of the muscles was left intact, and the temperature of the nerves was kept up by the heat of a lamp and irrigation with warm saline. Supramaximal "faradic" stimuli from an induction coil were applied to the proximal ends of the nerves, first to one, then to the other, and finally to both at once. Each series was repeated several times. The repetitions always proved consistent.

## RESULTS

On biopsy, the nerves were found to have become fully restored. Upon dissecting the arterial sleeve—which was never done until after the completion of the physiological tests—the Y-shaped union revealed perfectly smooth cylindrical surfaces with no evidence of the former lesion. In only two cases had the operation been defective. In one (R22), the distal stump had slipped from the sleeve so that only a tenuous strand of regenerated fibers had reached it (see Table 1), and in the other, the iliac sleeve over the proximal tibial stump had been so tight as to produce severe constriction, hence partial pressure block, of the nerve (9). The other 12 cases were fully successful.

In some cases, both tributaries joined the common distal trunk at an angle, in others the distal portion was aligned more or less straight with the axis of one or the other of the proximal stumps. This variability is attributable to variations in the distribution of tensions between the stumps, a straight connection between the distal stump and one of the proximal stumps indicating that the other proximal stump had been somewhat slacker. Such differences in orientation will undoubtedly have an influence on the relative share taken by the two fiber sources in the filling of the common distal stump. Fibers from both O and F thus arrive in the test muscle group in varying random combinations. The stimulation tests prove

that they have formed connections with the muscle fibers likewise entirely according to chance and without selectivity.

The earliest cases, studied after 7, 11, and 15 weeks, gave contractions of the plantar flexors after stimulation of either O or F. The muscles had only partially recovered and the tension records were inconclusive. The remaining 11 animals, studied at 16 weeks postoperative or later, all gave consistent records. These are listed in Table 1.

Considering that isometric tensions are roughly proportional to the num-

*Table 1. Relative contribution of peroneal and tibial nerves to reinnervation of plantar extensors as determined by isometric tensions after supramaximal nerve stimulation*

Specimen	Time p. op. days	Tension in grams after stimulation of			Inequality of innervation		Remarks
		n. tib.	n. per.	nn. tib. + per.	T > P	P > T	
R34	113	120	330	450		+	Heavy constrict. tib.
R31	119	780	200	930	+		
R32	119	80	440	500		+	
R33	119	10	630	650		+	
R30	120	450	710	900		+	
R26	124	470	80	?	+		
R28	124	300	100	380	+		
R29	124	80	230	300		+	
R25	125	450	110	500	+		
R27	125	100	450	550		+	Distal stump slipped out.
R22	129	100	30	130	+		
Average of all cases (omitting case R33)		290	270	520	+		

ber of muscle fibers innervated by the stimulated nerve, the table reveals the following facts:

(i) In all cases, both nerves, O and F, have taken part in the reinnervation of the plantar extensors.

(ii) The shares of O and F are always unequal, with the heavier contribution sometimes accounting for as much as 85 per cent of the total.

(iii) The heavier contribution is as likely to come from F as from O. Of 10 pertinent cases (omitting case R33 for reasons mentioned above), exactly half were innervated predominantly from O, the other half predominantly from F.

(iv) The fact that the muscles share the fibers of O and F wholly at random and with no preference for O is further illustrated by the grand averages of all cases, which are practically identical for both nerves. Since the tibial nerve normally contains at least 50 per cent more fibers than the peroneal, one could have expected a somewhat higher average contribution from the former, but evidently the chances have been about even for both.

(v) Simultaneous stimulation of O and F gave values equal to, or only slightly lower than, the sums of the values registered when the nerves were stimulated individually. This result is in line with the long-recognized fact that a single muscle fiber does not generally accept innervation from more than one motoneuron (1, 2, 11). The slight excess of the sum of tensions after separate stimulation over the tension produced by joint stimulation could be ascribed either to the presence of a few muscle fibers with double innervation, or, more probably, to certain mechanical peculiarities of isometric contractions recently pointed out by Löwenbach and Markee (4).

### DISCUSSION

In the described experiments, the tibial nerve, containing the original nerve supply of the plantar extensor muscles, and the peroneal nerve, containing only fibers foreign to them, were made to regenerate under as nearly identical conditions as possible and allowed to compete on equal terms for the reinnervation of these muscles. Under these conditions, any advantage of the original supply over the foreign supply should have become manifest, whether it be due to faster penetration, or to easier junction with the muscle fibers, or to an active inhibition of the foreign by the original fibers. Yet no trace of such differential has been found. The conclusion is that throughout the various steps of reinnervation—at entry into the distal stump; during passage through the distal stump; at entry into the muscle; during permeation of the muscle; and finally, in effecting terminal connections—all motor fibers, no matter what their former connections may have been, behave as equals. The chances for a muscle fiber to become innervated by a neuron from its original nerve or from a foreign nerve are the same, and in both cases the connections are effected with the same speed and readiness.

These unequivocal results invalidate the contention of Elsberg (1; p. 320), that "... the axis cylinders of the normal nerve to the muscle seem to be able to reestablish their former connections with the end plates or bulbs or to form new end organs more quickly or more powerfully than do those of a nerve which had belonged to a different muscle." The observations from which Elsberg had derived his conclusions could plausibly be explained by the fact that he allowed the fibers from the original nerve to reach the muscle by way of the preformed pathways of the old distal stump, while the foreign nerve was merely inserted in the intramuscular connective tissue, which obviously confronts regenerating fibers with greater difficulties, hazards and delays. Thus, the foreign fibers in his experiments were at a disadvantage for technical and not for constitutional reasons.

### SUMMARY

The problem of whether the original motor fibers of a muscle have any advantage over any other motor fibers in reinnervating that muscle was investigated by letting the tibial and peroneal nerves compete for reinnervation of the denervated plantar extensors. In 14 white rats, the proximal

tibial and peroneal stumps were joined to the distal tibial stump only. The junction was effected by means of a Y-shaped sleeve (Fig. 1) consisting of the reversed posterior end of the aorta, with the two iliac arteries serving as inlets for the two proximal nerves and funnelling the regenerating fibers into the common aortal trunk, which contained the distal tibial stump. Fibers from both sources thus travelled side by side and arrived in the muscles together.

After regeneration was completed, isometric tensions of the plantar extensors in response to supramaximal stimulation of the two nerve sources were determined (Table 1). The results proved that the fibers from both sources had reinnervated the muscles at random, and the original supply had no systematic advantage over the fibers of foreign origin. In half of the cases the original nerve innervated a greater share of the muscle fibers, and in the other half the foreign nerve took the greater share. The statistical average of all cases shows both sources to be of equal weight. The concept that there is any selectivity, absolute or relative, in the establishment of regenerative connections between motoneurons and muscle fibers is therefore contradicted by the facts.

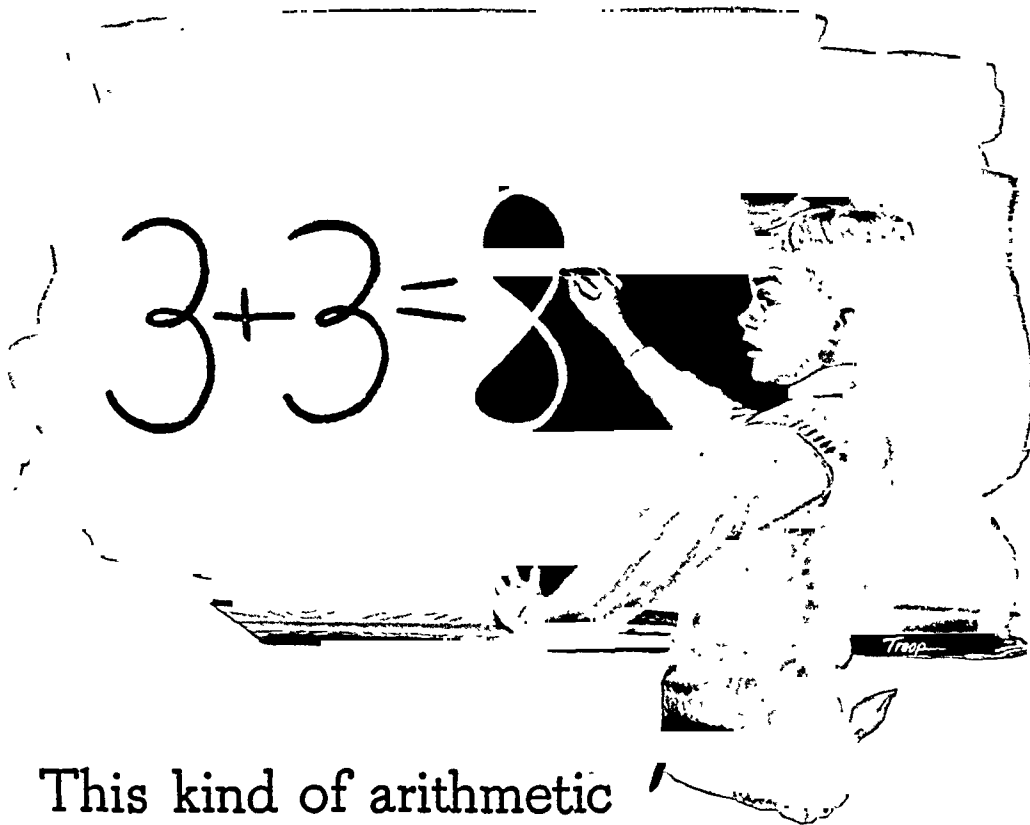
The experiments have also brought further confirmation of the fact that a single muscle fiber in general does not accept innervation from more than one motoneuron.

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# FACILITATION AND INHIBITION OF SPINAL MOTONEURONS

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STUDIES ON the duration of excitation and inhibition resulting at the motoneurons from the arrival of a single volley of impulses have suffered from the defect that such a volley seemingly was unattainable due to the intervention of internuncial bombardment. Clearly it is important to examine facilitation in circumstances that minimize, or preferably eliminate, internuncial activity as a factor in the conditioning of motoneurons. The description of experiments which follows is designed to show to what extent the attempt has been successful, and the results that have been obtained.

## CONDITIONS OF EXPERIMENT

The decapitate cat preparation has been employed, spinal section being accomplished through the dorsal atlanto-occipital membrane during ether anaesthesia, after which artificial respiration was commenced and the anaesthetic discontinued. Laminectomy was performed and the ventral roots of the lumbar enlargement sectioned, those of the seventh and eighth post-thoracic segments being prepared for the recording of reflex discharges. In the hind limb various combinations of muscle nerves belonging to the femoral, hamstring or sciatic groups were prepared for afferent stimulation. In this fashion, activity through two-neuron-arc reflex systems has been obtained, with functional selection made on the afferent limbs of the reflex pathways (cf. 12).

It has been found convenient to classify the afferent fibers of the A group (myelinated fibers of the somatic nerves) into three subdivisions (12). Group I, according to this classification, consists of the largest ( $20\mu$  to  $12\mu$  approximately) afferent fibers found only in the nerves of muscle. Group II includes the medium sized fibers ( $12\mu$  to  $6\mu$  approximately)—many in number in cutaneous nerves, sparsely present in muscle nerves. Group III coincides with the delta group. It is the group I fibers that form direct connections with motoneurons (11, 12, 13).

*Dorsal roots are not satisfactory for afferent "conditioning" stimulation.* Two-neuron-arc reflexes belonging to specific muscles or muscle groups may be obtained by stimulating appropriate dorsal roots and recording from the nerve to the muscle or muscles in question (22), or by stimulating a selected muscle nerve while recording from an appropriate ventral root (11, 12). Serious objections to the use of the former technique arise if the object is the conditioning of motoneurons. These are: (i) the afferent fibers of muscles of many actions, allied and antagonistic, are stimulated indiscriminately, and unavoidably so; and (ii) it is virtually impossible, in a dorsal root, to stimulate group I afferent fibers without the participation of group II afferent fibers in the stimulated fraction. For instance, Gasser and Graham (7) on stimulating dorsal roots found the negative intermediary potential appearing in threshold responses of the spinal cord. Again (11), discharges through multineuron reflex arcs have been seen when a dorsal root is stimulated at a strength just sufficient to evoke a two-neuron-arc discharge. Recently Eccles, too (5), has reported encountering this same difficulty.

*Muscle nerves as sources of afferent conditioning activity.* The chances of obtaining afferent volleys confined to group I fibers are increased if muscle nerves rather than dorsal roots are employed for afferent stimulation. Even so, certain precautions must be observed. It is important to stimulate with near-threshold shocks, otherwise indisputable evidence of internuncial activity frequently is encountered. In fact, analysis of the conditioning action of group I impulses may be furthered by observing the effect of deliberate, slight increase in the strength of the conditioning shocks (cf. Figs. 4, 5, 8, 9, 10). The fact that internuncial



activity of ipsilateral origin is predominantly excitatory to flexors and inhibitory to extensors (12) is an aid to identification.

The use of near-threshold shocks introduces some difficulty attending threshold "play." This is most serious in testing the early course of conditioning action in motor nuclei. However, since internuncial actions are delayed by slower afferent conduction in group II fibers and added synaptic time, the early course (but not the later course) of conditioning as revealed by weak shocks may be confirmed by the use of stronger shocks (15).

A group I volley stimulated in a muscle nerve possesses functional homogeneity, a most important factor in the present experiments.

*Test volleys.* From present knowledge there would appear to be no serious objection to the use of dorsal root volleys while recording from specific muscle nerves as two-neuron-arc test reflexes. In the experiments discussed here, this would be plausible only in certain situations dependent upon anatomy, as a result of the necessity for cutting ventral roots and preserving dorsal roots to provide for the conditioning activity. Therefore the practice of employing volleys selected on the afferent limb has been followed for the test system as

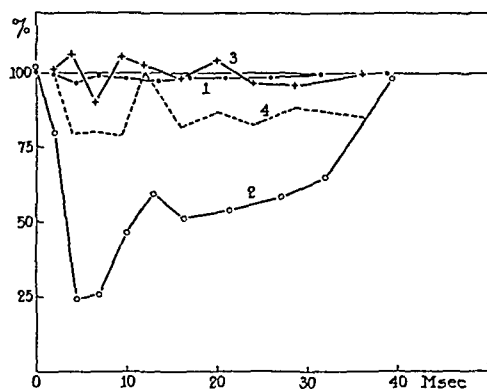


FIG. 1. Influence on reflex discharge of antidromic volleys in neighboring motoneurons. Gastrocnemius, nucleus. Curve 1: Antidromic volley maximal, reflex afferent volley maximal. Curve 2: Antidromic volley maximal, reflex afferent volley 12 per cent maximal. Curve 3: Antidromic volley 50 per cent maximal, reflex afferent volley 50 per cent maximal. Curve 4: Antidromic volley maximal, reflex afferent volley 50 per cent maximal. A strong antidromic volley coupled with a weak reflex volley is the requirement for significant depression of reflex discharge.

well as for the conditioning system. Strong stimulation of the test nerve, producing the maximum two-neuron-arc reflex attainable by stimulation of that nerve in isolation, has been used routinely. The occurrence of multineuron-arc reflex activity following the test two-neuron-arc response is of no consequence, and the advantage is gained that random variability is less than it is with submaximal reflexes.

*Minimizing random variation.* Spontaneous fluctuation in the excitability of the preparation has proved something of a difficulty. The use of strong test shocks is of some benefit, but it has been found advisable, for each point on the experimental curves—examples of which are illustrated—to record a number of observations, with conditioned and control records obtained in alternation. The average is struck for conditioned and control values and the two compared.

*Influence of motoneuron discharge upon the excitability of neighboring motoneurons.* Since Renshaw (21) demonstrated that antidromic volleys occupying some motoneurons can, in some circumstances, alter the excitability of neighboring motoneurons either in the direction of enhancement or depression, it is of obvious importance to assess the possible influence of comparable effects in the present experiments. Since the ventral roots have been severed, one does not have to consider the action of antidromic volleys as such, but, embracing for the moment the assumption that firing motoneurons would have comparable actions whether fired antidromically or orthodromically, the possibility of interaction exists. Accordingly, some of Renshaw's experiments have been repeated with general conditions, such as the type of preparation, absence of anaesthesia and site of stimulation, adjusted to conform more closely with those obtaining in the experiments to be described. Illustrative interaction curves are presented in Figure 1, in which is examined the effect upon a reflex discharge into the nerve of one head of gastrocnemius of an antidromic volley from the

nerve to the other head of gastrocnemius. The curve represented by dots was obtained by employing maximal antidromic volleys and maximal reflex volleys. It should be emphasized that the reflex was maximal in the sense that increased stimulation of the dorsal root did not further increase the test reflex, not in the sense that the reflex did not increase by virtue of facilitation by convergent activity of similar origin. A slight depression is noted, the maximum amounting to 3.5 per cent at 4.5 msec. separation of antidromic and orthodromic volleys.

Reduction of the test reflex to approximately 12 per cent of its former size, the antidromic volley remaining maximal, resulted in a change in the interaction curve to that designated by circles. At maximum a depression of reflex response amounting to 75 per cent is realized. The third curve of Figure 1, that indicated by crosses, was obtained by a combination of antidromic and orthodromic volleys, both types being about 50 per cent of maximum. The deviation of the points, insofar as it may be significant, is about equally in the direction of enhancement and depression. The third curve may be compared with the fourth, represented by a broken line, to obtain which the antidromic volley was increased to maximum, the orthodromic volley being retained at the same size, to yield a half-maximum reflex.

It is concluded from the data presented in Figure 1 that the conditions for significant interaction between motoneurons, even those pertaining to the nucleus of a single muscle, are: (i) powerful activation, in terms of numbers, of the motoneurons acting together as the agent; and (ii) feeble activation of the responding test reflex system. Since, in the experiments to be considered in this paper, the discharge of motoneurons by the conditioning volley is at most very small compared with that realized by antidromic activation, and since the test reflexes are strong by comparison with those that are significantly altered by antidromic volleys in nearby motoneurons, further consideration of motoneuron interaction is unnecessary for the purpose at hand.

## RESULTS

*I. Facilitation of motoneurons by the direct action of primary afferent impulses.* Although reflex discharges through arcs of two neurons return only to the muscle nerve from which they arise (12), it has been found that mutual facilitation obtains between closely allied two-neuron-arc pathways. For example, group I volleys arising in the nerves to different parts of biceps femoris posterior interact at the biceps motor nucleus. Similar facilitatory interaction occurs between the two heads of gastrocnemius (also noted by Eccles (5), to whom credit is due for the prior demonstration of this interaction), and even between semitendinosus and biceps femoris posterior, two distinct although synergic muscles. The fact of such interaction provides the required situation for a study of the summation period at the motoneurons.

Figure 2 presents records obtained from an experiment in which two nerve branches to biceps were employed for stimulation by single shocks, reflex discharge being recorded from the eighth post-thoracic ventral root. The branches were of unequal size, and the shock to the smaller, called here the conditioning shock, evoked an afferent volley that was insufficient to secure a reflex discharge. The shock to the larger branch, here called the test shock, resulted in the two-neuron-arc reflex discharge to be seen, in isolation, in records A and O of Figure 2. The two afferent paths were of equal length so that, with synchronous shocks, the two afferent volleys reached the spinal cord in concert. Record B illustrates the result of such synchronous stimulation of the two branches. The reflex response is increased three-fold. As the test shock falls increasingly later with respect to the conditioning shock

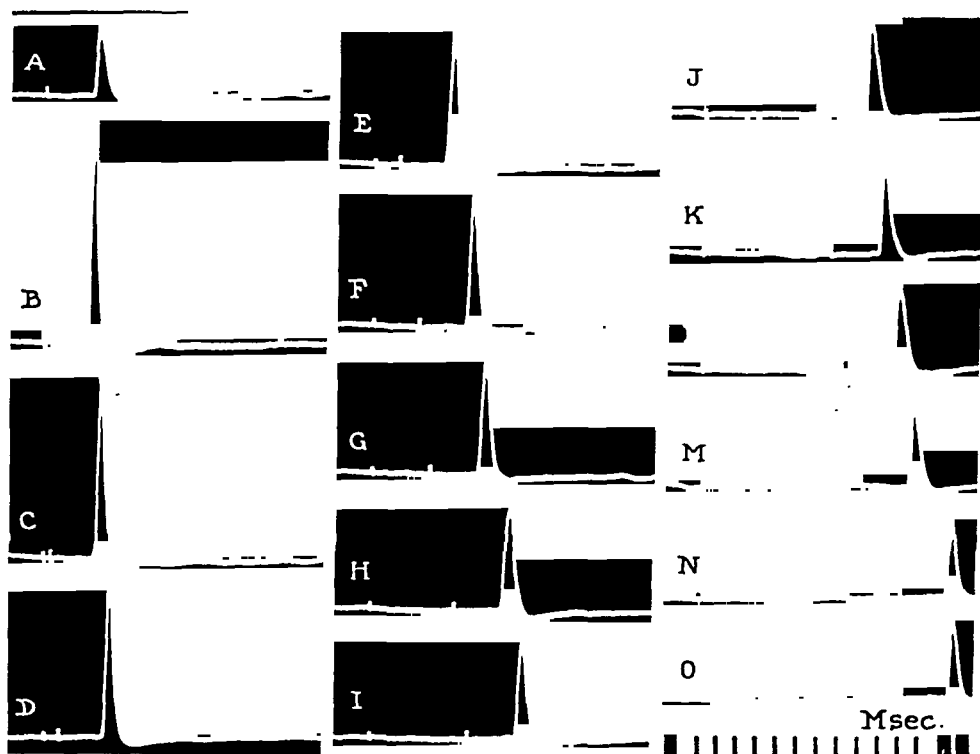


FIG. 2. Facilitation of biceps reflex by afferent volley from another branch of biceps nerve. A, O: biceps reflex in isolation. B-N: Biceps reflex tests effect in the biceps nucleus of the afferent conditioning volley, coincident with (B), and at various intervals after arrival of the conditioning volley. The conditioning volley evokes no reflex response. Time: msec.

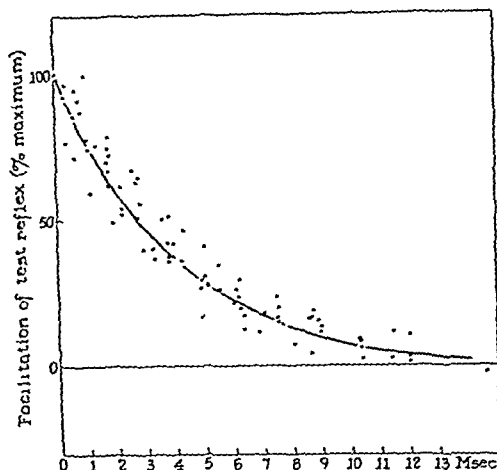
(Records C to N of Fig. 2), facilitation of the test reflex declines progressively, at first rapidly and then more slowly, over a time course extending in the figure to 10 msec.

A better indication of the time course of facilitation in motor nuclei may be found in Figure 3, in which are plotted the results obtained in seven individual experiments on as many preparations. In four of the experiments facilitation in flexor nuclei was examined, either by fractionating the afferent inflow from biceps or by causing interaction between volleys arising in the nerves of semitendinosus and biceps. The three other experiments, performed in examination of facilitation in extensor nuclei, record the interaction at the motoneurons of volleys arising in the nerves to the two heads of gastrocnemius. Naturally the intensity of facilitation varies from one experiment to another, hence the points from the individual experiments have been scaled so that they coincide at the time of maximum facilitation.

It will be seen from Figure 3 that facilitation is maximal if the two volleys

arrive simultaneously at the motoneurons, and that it decays over a relatively prolonged time course that can be represented by an exponential curve, as drawn, having a value for  $1/e$  of approximately 4 msec. Furthermore, there are no apparent discontinuities in the curve of facilitation to suggest the intervention of relayed activity in the conditioning of the test reflexes, and finally, the points obtained by experiment on flexor and extensor nuclei show satisfactory correspondence. From the evidence presented it appears

FIG. 3. Facilitation of motoneurons by impulses in primary afferent fibers. Points from seven experiments, scaled on the ordinates to coincide at the time of maximum facilitation. Relative facilitation, expressed in per cent maximum, is plotted as a function of time. The plotted curve is an exponential regression having successive half-values at 2.8, 5.6, 8.4, 11.2 and 14 msec. In four experiments facilitation in flexor nuclei was examined; the remaining three were concerned with extensor nuclei. Individual experiments on facilitation in flexor and extensor nuclei are to be found in Figs. 4 and 5 respectively.



that the facilitation curves, and hence the summation periods at the motoneurons, are similar whether the motoneurons belong to a flexor or to an extensor nucleus. The summation period defined by the curve in Figure 3, therefore, would seem to be a general property of the synapses of two-neuron-arc systems of the spinal cord. It may be noted that the curve of Figure 3 is drawn according to a regression having successive half-values at 2.8, 5.6, 8.4, 11.2, and 14 msec. In all of the succeeding figures the same curve has been drawn. Divergences from reasonable agreement with the function and curves representing the conditioning of motoneurons by internuncial activity have been plotted by simple joining of the experimentally determined points. All solid line plots represent experimental observations, broken line curves being extrapolations according to expectation.

*II. Effect in a flexor nucleus of increasing the conditioning volley.* One naturally is reluctant to accept prolonged excitatory action such as that described in connection with Figures 2 and 3 as resulting from single synchronous volleys of impulses unaided by the reverberant activity of parallel chains of interneurons. A way to assess the "purity" of the action is to observe the effect within a motor nucleus of deliberate addition, into the conditioning volleys, of some group II impulses; and by this means, adding known internuncial activity to the total conditioning agent at the motoneurons tested. Figure 4 illustrates the result of an experiment dealing with a flexor nucleus, in which are compared the conditioning effects of afferent

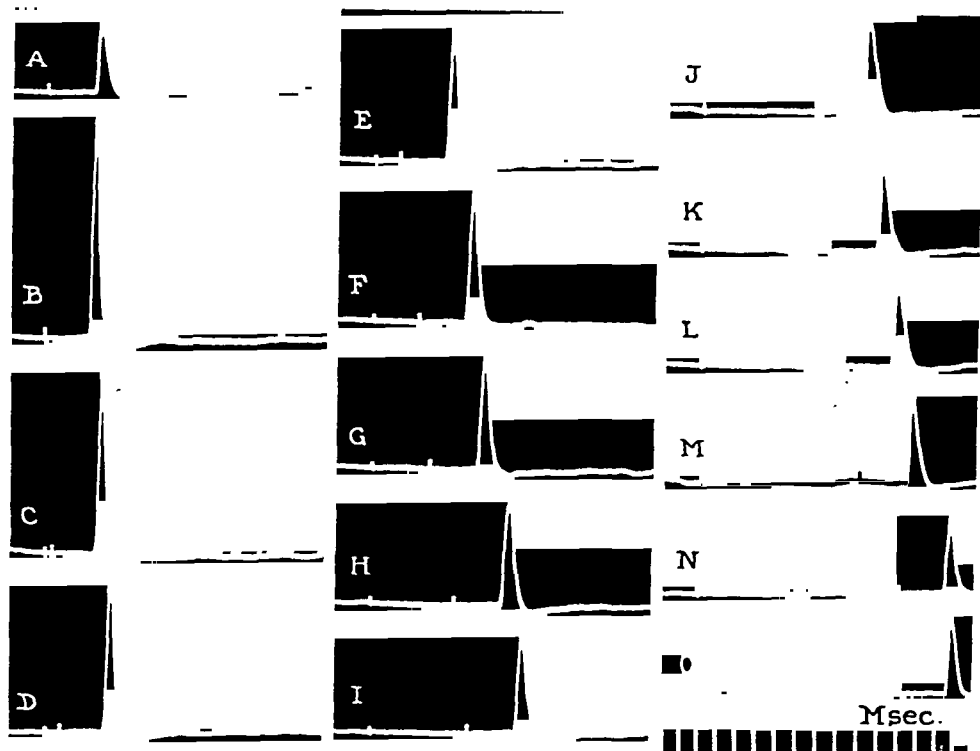


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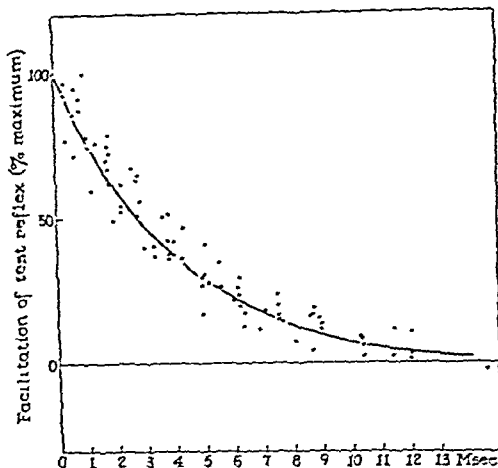
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volleys of two strengths. The conditioning stimulation was applied to the nerve of semitendinosus, the test stimulation to a branch supplying biceps. The experimental points, indicated by dots in Figure 4, were obtained by the use of a shock 1.1 threshold for the (inconstant) appearance of reflex response in the conditioning system. The test stimulus was strong. The experimentally determined points are represented satisfactorily by the regression curve as drawn. In order to obtain the curve represented in Figure 4 by the crosses, the conditioning shocks were increased to 1.4 threshold, the test shocks remaining at the same strength. One will notice that no appreciable change in the course of facilitation has taken place in the first 1.5 msec. However, at about this stimulus interval a sharp break in the curve occurs

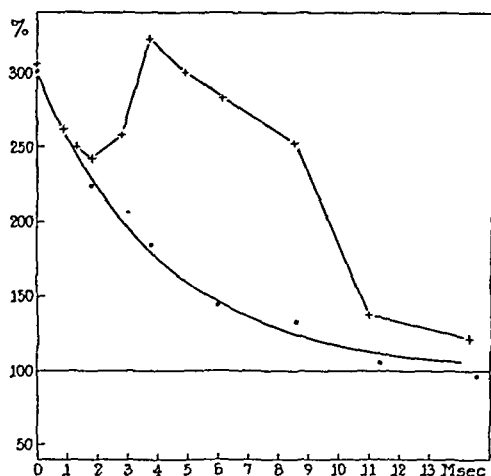


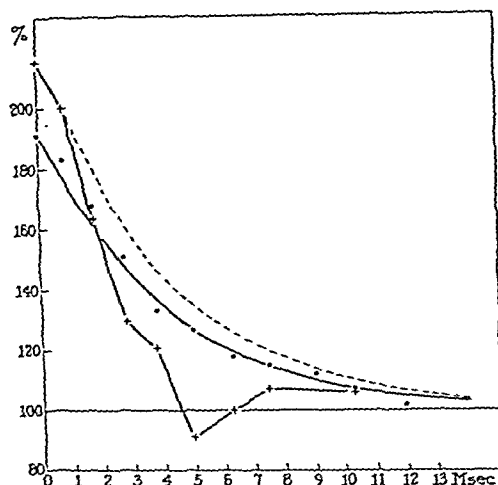
FIG. 4. Facilitation of biceps reflex by afferent volleys in semitendinosus nerve. Amplitude of the test reflex, expressed as per cent of control amplitude, is plotted as function of the time interval between conditioning and test volleys. To obtain the curve represented by dots, conditioning volleys of near reflex threshold strength were used. Stronger conditioning volleys caused the appearance of a second period of facilitation (crosses).

and thereafter a second period of facilitation supervenes. Since the test object is in this instance a flexor nucleus, and since the action of interneurons in an ipsilateral system is predominantly excitatory to flexors (12), the observed result of increasing in strength the conditioning volleys is entirely in accord with expectation on the proposition that conditioning by internuncial activity has been added to conditioning by the primary afferent fibers that course directly to the motoneurons. The latency for the internuncial action, measured approximately by the time to the break between the two curves, is accounted for by additional afferent conduction time required for group II fibers (12) and additional synaptic delay.

*III. Effect in an extensor nucleus of increasing the conditioning volley.* It is appropriate to investigate the effect of slight increase in the size of conditioning volleys where the object of study is an extensor rather than a flexor nucleus. Figure 5 presents results that have been obtained in experiment designed for this purpose. Conditioning shocks were applied to the nerve of one head of gastrocnemius, test shocks to the other. The points indicated

by dots were obtained with the use of weak conditioning volleys, and may be represented by the usual regression curve as drawn. The points indicated by crosses were obtained by repeating the experiment utilizing somewhat stronger conditioning volleys. It is obvious, by comparison of the earliest points of the two curves, that the weaker of the two strengths of conditioning shock was submaximal for the direct fibers of the nerve. Accordingly, we find the curve obtained by the use of the stronger shocks beginning higher. The broken line, then, is an extrapolation indicating the curve of facilitation to be expected as a result of the stronger conditioning volleys, provided no change other than increase in the group I afferent volleys took place. In actual fact this curve is representative of the experimental points during the

FIG. 5. Facilitation of reflex pertaining to one head of gastrocnemius by afferent volleys in nerve to the other head. Ordinates and abscissae as in Fig. 4. The dots represent points obtained by the use of weak conditioning volleys. On strengthening the conditioning volleys slightly, the points identified by crosses were obtained. Initially, by use of the stronger conditioning volleys, facilitation is increased but the subsequent course is interrupted by secondary inhibition. Broken line: extrapolation to show expected course of facilitation if not so interrupted.



first msec., but, thereafter, the experimental points diverge in the direction of inhibition. Since the object of study is an extensor nucleus and since ipsilateral internuncial activity is predominantly inhibitory to extensor neurons, the conditioning curve obtained by the use of the stronger conditioning shocks is interpreted as the resultant of a combination of excitation by direct action of primary afferent fibers and inhibition by the action of interneurons.

IV. *Inhibition of motoneurons.* Afferent fibers that yield inhibition of motoneurons by direct action (10) are known to be indistinguishable, on the basis of elementary properties, from those that yield excitation (11). Therefore they too are large afferent fibers of muscle origin. Inasmuch as afferent fibers of this group arising in heads of a single muscle, or in closely allied synergists, mutually facilitate the excitatory actions of each other, one might reasonably expect inhibitory interaction to result between volleys arising in the afferent fibers of antagonists. The expectation is based on the supposition that direct inhibition by primary afferent fibers represents the stretch-evoked inhibition of antagonists described by Sherrington (24) and by Lid-



volleys of two strengths. The conditioning stimulation was applied to the nerve of semitendinosus, the test stimulation to a branch supplying biceps. The experimental points, indicated by dots in Figure 4, were obtained by the use of a shock 1.1 threshold for the (inconstant) appearance of reflex response in the conditioning system. The test stimulus was strong. The experimentally determined points are represented satisfactorily by the regression curve as drawn. In order to obtain the curve represented in Figure 4 by the crosses, the conditioning shocks were increased to 1.4 threshold, the test shocks remaining at the same strength. One will notice that no appreciable change in the course of facilitation has taken place in the first 1.5 msec. However, at about this stimulus interval a sharp break in the curve occurs

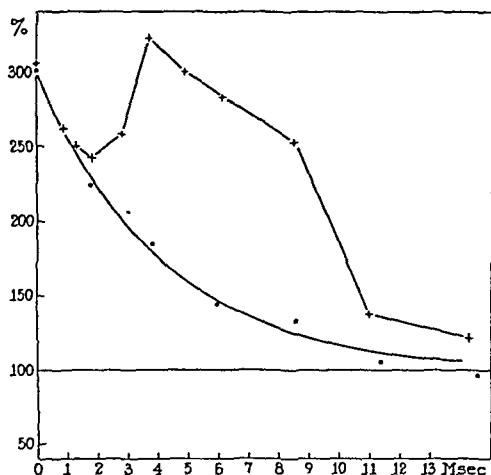


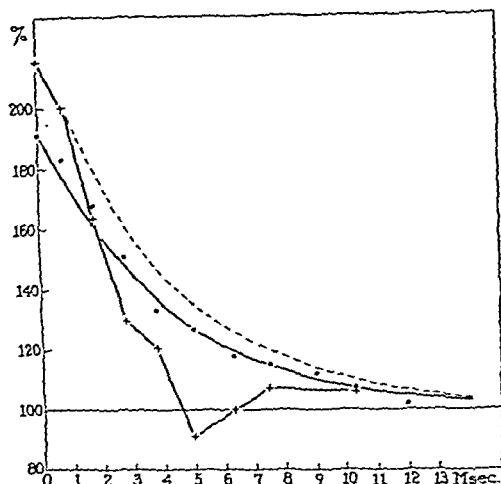
FIG. 4. Facilitation of biceps reflex by afferent volleys in semitendinosus nerve. Amplitude of the test reflex, expressed as per cent of control amplitude, is plotted as function of the time interval between conditioning and test volleys. To obtain the curve represented by dots, conditioning volleys of near reflex threshold strength were used. Stronger conditioning volleys caused the appearance of a second period of facilitation (crosses).

and thereafter a second period of facilitation supervenes. Since the test object is in this instance a flexor nucleus, and since the action of interneurons in an ipsilateral system is predominantly excitatory to flexors (12), the observed result of increasing in strength the conditioning volleys is entirely in accord with expectation on the proposition that conditioning by internuncial activity has been added to conditioning by the primary afferent fibers that course directly to the motoneurons. The latency for the internuncial action, measured approximately by the time to the break between the two curves, is accounted for by additional afferent conduction time required for group II fibers (12) and additional synaptic delay.

*III. Effect in an extensor nucleus of increasing the conditioning volley.* It is appropriate to investigate the effect of slight increase in the size of conditioning volleys where the object of study is an extensor rather than a flexor nucleus. Figure 5 presents results that have been obtained in experiment designed for this purpose. Conditioning shocks were applied to the nerve of one head of gastrocnemius, test shocks to the other. The points indicated

by dots were obtained with the use of weak conditioning volleys, and may be represented by the usual regression curve as drawn. The points indicated by crosses were obtained by repeating the experiment utilizing somewhat stronger conditioning volleys. It is obvious, by comparison of the earliest points of the two curves, that the weaker of the two strengths of conditioning shock was submaximal for the direct fibers of the nerve. Accordingly, we find the curve obtained by the use of the stronger shocks beginning higher. The broken line, then, is an extrapolation indicating the curve of facilitation to be expected as a result of the stronger conditioning volleys, provided no change other than increase in the group I afferent volleys took place. In actual fact this curve is representative of the experimental points during the

FIG. 5. Facilitation of reflex pertaining to one head of gastrocnemius by afferent volleys in nerve to the other head. Ordinates and abscissae as in Fig. 4. The dots represent points obtained by the use of weak conditioning volleys. On strengthening the conditioning volleys slightly, the points identified by crosses were obtained. Initially, by use of the stronger conditioning volleys, facilitation is increased but the subsequent course is interrupted by secondary inhibition. Broken line: extrapolation to show expected course of facilitation if not so interrupted.



first msec., but, thereafter, the experimental points diverge in the direction of inhibition. Since the object of study is an extensor nucleus and since ipsilateral internuncial activity is predominantly inhibitory to extensor neurons, the conditioning curve obtained by the use of the stronger conditioning shocks is interpreted as the resultant of a combination of excitation by direct action of primary afferent fibers and inhibition by the action of interneurons.

IV. *Inhibition of motoneurons.* Afferent fibers that yield inhibition of motoneurons by direct action (10) are known to be indistinguishable, on the basis of elementary properties, from those that yield excitation (11). Therefore they too are large afferent fibers of muscle origin. Inasmuch as afferent fibers of this group arising in heads of a single muscle, or in closely allied synergists, mutually facilitate the excitatory actions of each other, one might reasonably expect inhibitory interaction to result between volleys arising in the afferent fibers of antagonists. The expectation is based on the supposition that direct inhibition by primary afferent fibers represents the stretch-evoked inhibition of antagonists described by Sherrington (24) and by Lid-

dell and Sherrington (9). Experimental result is in accord with this expectation.

Figure 6 presents records from an experiment in which a shock for conditioning, small in size, was applied to the deep peroneal nerve, and a strong test shock was applied to the combined gastrocnemius nerves. Recordings were made from the eighth post-thoracic ventral root. The afferent paths were of unequal length, that for the conditioning volleys being the longer.



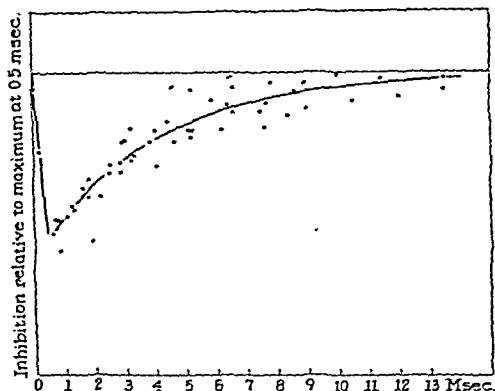
FIG. 6. Inhibition of gastrocnemius two-neuron-arc reflex by weak afferent volleys in deep peroneal nerve. A, M: control records of gastrocnemius reflex in isolation. B-L: conditioning effects when conditioning and test shocks are separated by various intervals. Since the afferent paths were of unequal length shock interval does not give volley interval. The necessary correction is given in the text. Time: msec.

For this reason a correction must be made to the shock interval to allow for differential conduction time. It was found that the conditioning and test volleys arrived at the spinal cord simultaneously when the test shock followed the conditioning shock by approximately 0.4 msec. The conditioning shock evoked no reflex response in the eighth post-thoracic ventral root, whereas the test shock evoked, in that root, the two-neuron-arc reflex response to be seen in observations A and M of Figure 6. For observation B the two volleys (but not the shocks) were separated by an interval of approximately 0.2 msec. Inhibition is well established. As the test shock falls

progressively later with respect to the conditioning shock (C to L of Fig. 6), inhibition of the test response increases rapidly to a maximum and then regresses slowly over a prolonged time course.

A more complete representation of the course of inhibition may be obtained by inspection of Figure 7, in which are plotted the results of four experiments dealing with inhibitory interaction. In three of the experiments inhibition in a flexor nucleus was measured; in the fourth, the reverse situation was examined. Since the degree of inhibition varied from experiment to experiment, the experimental points have been scaled to coincide at 0.5 msec. on the abscissa. This point for coincidence was chosen for two reasons:

FIG. 7. Inhibition of motoneurons by impulses in primary afferent fibers. Experimental points from four experiments are included; the points are scaled to coincide at a conditioning volley-test volley interval of 0.5 msec. The ordinates therefore relate the degree of inhibition, in per cent of maximum, to the time interval between volleys (on the abscissae). Three experiments were concerned with flexor nuclei, the remaining with an extensor nucleus. Curves obtained in individual experiments may be found in Figs. 8 and 9.



inhibition is maximal at approximately this time and internuncial activity, if such were present, would not influence the scaling of the points. The regression curve that has been drawn through the points in Figure 7 is the same as that drawn for facilitation in Figure 3: it decays to successive half-values of 2.8, 5.6, 8.4, 11.2 and 14 msec.

The plotted curve in Figure 7 is a fair representation of the experimental points: it shows that inhibition begins with synchronous arrival of the interacting volleys at the spinal cord (9), that there is an incremental phase of approximately 0.5 msec. duration, and that inhibition regresses over a prolonged time course that may be represented by an exponential curve having a value for  $1/e$  of approximately 4 msec. The inhibitory period is similar whether examined in flexor or in extensor nuclei, and therefore would seem to be a general property of the inhibitory junctions in the motor nuclei.

V. *Effect in a flexor nucleus of increasing conditioning volley.* As in the study of facilitation, it is possible to demonstrate that sharp departures from the simple inhibitory curve result from the deliberate instigation of internuncial activity by increased conditioning volleys. Figure 8 presents an experiment in which a flexor two-neuron-arc reflex was conditioned by volleys arising in the nerves to the antagonist extensor. The influence on the test reflex of two strengths of afferent conditioning volleys is compared. The conditioning stimulation was applied to the nerves of gastrocnemius, the

test stimulation to the nerve of tibialis anterior. Records were obtained from the seventh post-thoracic ventral root. The experimental points indicated by dots were obtained by the use of the weaker conditioning volleys. These points are represented satisfactorily by the solid line regression curve as drawn. The experimental points indicated by crosses resulted from conditioning with the slightly stronger afferent volleys. It is apparent from a consideration of Figure 8 that the weaker conditioning shocks were not maximal

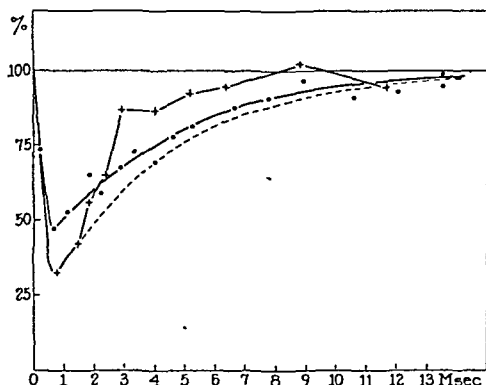


FIG. 8. Inhibition in a flexor nucleus, and interruption of inhibition by excitation of internuncial origin. To obtain the points identified by dots, weak gastrocnemius nerve afferent volleys were used to condition the reflex of tibialis anterior. On strengthening the conditioning volleys (crosses) the primary inhibition was increased, but after a brief interval is interrupted by facilitation of flexor reflex origin. Broken line: extrapolation to show inhibitory curve to be expected if stronger conditioning volleys had had no effect other than increasing primary inhibition.

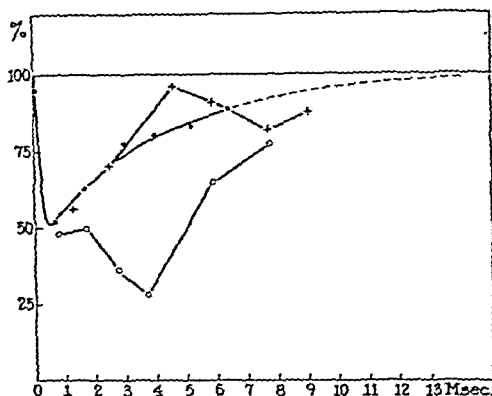
for the group I afferent fibers of the gastrocnemius nerve since on increasing those shocks the inhibition in the nucleus of tibialis anterior, at the shortest shock intervals, was increased. The broken line regression curve is drawn by extrapolation from the first two points represented by crosses, in order to indicate the curve of inhibition to be expected if, as a result of the increased conditioning shocks, no change other than increase in the number of group I fibers stimulated took place. The experimentally determined points diverge from this curve at an interval of approximately 1.5 msec. between volleys, and do so in the direction of facilitation. The latency for the beginning of divergence and the direction of the divergence—since the test system was a flexor two-neuron-arc reflex—are consistent with the view that internuncial activity was added by the stronger conditioning shocks to the direct action of the primary afferent fibers, and that the internuncial activity was responsible for the break in the resulting conditioning curve.

VI. *Effect in an extensor nucleus of increasing conditioning volley.* Conditioning effects in the gastrocnemius (extensor) nucleus evoked by afferent volleys arising in the deep peroneal (flexor) nerve reveal a more complex situation than has yet been encountered, but one that is entirely consistent with the known reflex behaviour of gastrocnemius muscle. One will recall the study made by Denny-Brown (cf. 1, p. 73) of the comparative behaviour of gastrocnemius and soleus in response to stimulation of afferent nerves. Particularly noteworthy is the fact that stimulation by weak shocks of the peroneal nerve, acting on a background of stretch reflex in gastrocnemius

and soleus, results in an increased contraction of the former coincident with relaxation, from the stretch reflex plateau, of the latter. Stronger stimulation of the peroneal nerve caused both muscles to relax. The experiments illustrated in Figures 9 and 10 should be interpreted in the light of those findings.

The experimental points (identified by dots in Fig. 9) were obtained by the use of weak shocks to the deep peroneal nerve delivered for the purpose

FIG. 9. Inhibition in an extensor (gastrocnemius) nucleus resulting from afferent volleys in deep peroneal nerve. The curves indicate various admixtures of primary inhibition by the direct action of primary afferent fibers, secondary facilitation and secondary inhibition, resulting from internuncial action. Further details in text.



of conditioning a gastrocnemius two-neuron-arc reflex. The points were obtained in the order of increasing shock interval, and may be represented by the usual regression curve. Next in order the points indicated by crosses were obtained, without deliberately increasing the conditioning volleys. These points indicate a good correspondence at short intervals, but a degree of divergence thereafter, first in the direction of facilitation, subsequently in the direction of inhibition. Deliberate slight increase in the conditioning volleys then resulted in the points identified by circles. These last points reveal the appearance of a second phase of inhibition. The curves represented in Figure 9 by crosses and circles are interpreted as indicating the conditioning of gastrocnemius motor nucleus by a combination of direct inhibitory action of primary afferent fibers, together with varying admixtures of excitatory and inhibitory internuncial activity.

Figure 10 illustrates another experiment in which the conditioning effect on gastrocnemius nucleus of volleys in the deep peroneal nerve was examined. The first curve (indicated by dots) was obtained by the use of conditioning shocks obviously submaximal for the initial inhibition, since this inhibition was increased by increasing the conditioning shocks. The shocks, therefore, were submaximal for the group I fibers of the deep peroneal nerve; and yet the curve shows, after the first 1.5 msec., a distinct deviation in the direction of facilitation. Slightly stronger conditioning stimulation increased the direct inhibitory action to maximum (curve indicated by crosses), and the later deviation is somewhat less in the direction of facilitation. Finally

(curve indicated by circles), by virtue of further increase in the conditioning stimulation, the secondary deviation is strongly in the direction of inhibition, there being no further intensification of the primary inhibitory action.

Consider now, in connection with Figures 9 and 10, the three effects in gastrocnemius nucleus resulting from afferent volleys in the deep peroneal nerve: primary inhibition, secondary facilitation and secondary inhibition. A distinct threshold difference distinguishes the afferent fibers responsible for the first two effects and those responsible for the last mentioned. It seems quite clear, therefore, that secondary inhibition results from the stimulation of group II fibers, that it represents the extensor-inhibitory component of

the flexor reflex and that it is the result of internuncial activity. The origin of secondary facilitation is less certain, not in the sense that there is any reason to suppose that it does not result from internuncial activity, but in the sense that experiment has not indicated clearly the classification to which the executant afferent fibers belong. Bearing in mind the vagaries of stimulation, the overlapping thresholds for primary inhibition and secondary facilitation in the reflex system being considered may not indicate overlapping properties of the afferent fibers giving rise to the

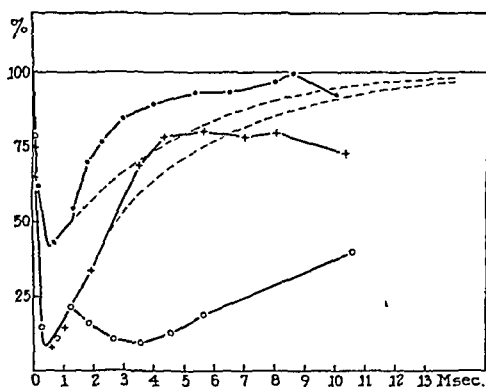


FIG. 10. As in Fig. 9, from another experiment. Full description in text.

two effects. On the other hand, it is so difficult to extricate the primary inhibition in gastrocnemius nucleus from the secondary facilitation that one must entertain the possibility that secondary facilitation in this nucleus represents the activity of certain interneurons stimulated into activity by collaterals of the group I afferent fibers of the deep peroneal nerve. For that matter, if one considers the weak afferent stimulation that is necessary in order to realize simple conditioning curves in the light of Cajal's demonstration of collaterals given off to the internuncial regions by the "reflexo-motor collaterals," it may be that isolated direct action at the motoneurons appears only when the supposed volleys entering the internuncial pools by way of these collaterals are of intensity insufficient to discharge interneurons. This possibility does not modify the main conclusions that the simple conditioning curves represent the unaided conditioning activity—excitatory and inhibitory at proper loci—of primary afferent fibers, and that the bulk of the secondary conditioning effects, following strictly the reciprocal pattern of the flexor reflex, results from the stimulation of group II afferent fibers that are known to mediate the flexor reflex (12).

## COMMENT

VII. *Considerations relating to excitation of motoneurons.* The "synaptic potential." With synaptic transmission in the spinal cord blocked completely by deep anaesthesia, Eccles (5) has shown that an afferent volley evokes in an appropriate ventral root a potential change that has negative direction at the recording lead adjacent to the spinal cord. As recorded, this "synaptic potential" has a rising phase of 2.5 msec. duration, followed by an approximately exponential regression falling to  $1/e$  in 7.5 msec. Assuming that the site of origin of the synaptic potential is central to the point of electrode contact, allowance should be made for changes suffered in the course of electrotonic spread to the ventral root from the presumed site of origin. This having been done, it is likely that a reasonable degree of agreement would obtain between the decay of facilitation at the motoneurons and the decay of the synaptic potential. One may assume a correlation between the two phenomena.

It is of interest that Eccles finds the exponential regression of the synaptic potential interrupted by a "hump" if the anaesthesia is not sufficiently deep to block all internuncial discharge. This effect is comparable to the break from exponential regression in the facilitation curve if a test reflex in the unanaesthetized preparation is conditioned by afferent volleys slightly larger than critical size (e.g., Fig. 4). Thus Eccles has achieved by deep anaesthesia and strong volleys what is achieved here by the use of feeble volleys in muscle nerves: the avoidance of internuncial activity. Obviously the latter method would not be very satisfactory for study of the synaptic potential, which, because of the necessary condition, would be impractically small.

*On the course of excitation at the motoneuron.* Focussing one's attention solely upon the action at the motoneurons of a single synchronous "excitatory" volley, uncomplicated by the restimulating action of interneurons, it appears necessary now squarely to face the question of whether or not the excitatory event at the synapse contains two (at least) components: one brief, powerful, and capable of adequate stimulation of the motoneurons; the other of longer duration, not so powerful, capable of facilitating the action of the first mentioned component, but not of itself capable of discharging motoneurons. The first component would be identified as the detonator action (2, 3, 18); the latter, for reasons that will become apparent as the argument develops, may be called "residual facilitation."

The train of evidence that led to the concept of a brief excitatory or detonator action is well known and has been summarized extensively (2, 18); it need not be reiterated here. The existence, at least in significant measure, of a second excitatory component of synaptic excitation in the central nervous system has remained problematical until the recent demonstrations by Eccles, and in the present paper. Before the demonstration of a prolonged summation period of significant proportions it was unnecessary,



as Lorente de Nó stated (18), to assume excitatory actions other than detonator action in theoretical discussion of the then known properties of synaptic transmission. At the present time the alternatives are to assume two components in synaptic excitation, or to reject the concept of detonator action (4, 5). For a number of reasons the latter course seems precipitate, if not unjustified.

The postulation of two components of synaptic excitation does not demand the assumption of elementary properties that have no analogues in peripheral nerve. There are in published form at least three lines of evidence to support the assertion: (i) impulses arriving at a block in peripheral nerve establish two gradients of negativity at the margin of the block, one of spike-like dimensions, the other (called residual negativity) having a more enduring character (18). Facilitation across the block reflects faithfully the potential sequence, and so too exhibits two components. (ii) Lorente de Nó and Davis (19) have described two components of electrotonus in nerve, one fast, the other slow. (iii) Marrazzi and Lorente de Nó (20) have shown two components in the membrane potential change of fibers influenced by the passage of impulses in neighboring fibers. One important consideration in each of these three situations in peripheral nerve is the fact that the fast components exhibit a sharp spatial decrement, whereas the slow components spread for considerable distance. The first of the three situations is particularly important for present considerations, for Lorente de Nó (18) has employed the block in nerve as a model of synaptic transmission that makes adequate provision for the assumption of two successive gradients of negativity at the synaptic knobs: detonator negativity and residual negativity. Detonator negativity would be characterized by brief duration, powerful action and sharp spatial decrement. Residual negativity would be more prolonged, less powerful and more widespread. Indeed, it seems likely that the known differential decrement of fast and slow components, when applied to synaptic events, might be a partial explanation for the fact that the synaptic potential recorded at the ventral root does not indicate the presence of a detonator negativity. Clearly then, one should not preclude the existence of detonator negativity or detonator action from considerations of the synaptic potential as recorded.

*Problem of facilitation without discharge.* One observation of particular concern in this paper is well accommodated by the assumption of two components in synaptic excitation. Afferent volleys in group I fibers arising in one fraction of a muscle give rise to two-neuron-arc discharges reflecting into the motor fibers of that fraction of the muscle but not elsewhere (12), yet the motoneurons pertaining to the rest of the muscle and its immediate synergists are facilitated for a considerable period of time. Admitting the assumption of the first component of excitation, it is a sufficient explanation for the absence of "cross discharge" to suppose that the afferent fibers from one fraction of a muscle have synaptic knobs on the motoneuron somata of the other fractions, but not at any point in dense clusters (17, 14). A second

component would account for "cross facilitation" having the properties described. Rejection of the assumption of the first component of excitation, because of the nature of the second component, would remove the anatomical and functional bases for an understanding of the absence of "cross discharge."

In view of these considerations and others, such as the brief, relatively fixed duration of synaptic delay (16) that are not of immediate concern here, it is concluded that the present experiments demonstrate, and describe some of the properties of, a second phase of summation at synapses in the central nervous system.

*VIII. Considerations relative to inhibition.* The experiments described in this paper indicate the existence of an unitary inhibitory process set into operation by ordinary nerve impulses travelling in afferent nerve fibers that are indistinguishable from those that excite motoneurons (10, 11). The inhibitory process as revealed at the motoneurons is characterized by an incremental phase of some 0.5 msec. duration followed by an essentially exponential regression falling to  $1/e$  in approximately 4 msec. The continued influence of the inhibitory action evoked by a synchronous volley is recognizable in experiment for 14 to 15 msec. The inhibitory process is active in the sense that afferent impulses, of inhibitory effect at a given locus, exert their action directly upon the structures, the excitability of which is depressed—the effect not depending in any way upon refractoriness anywhere in the system. The inhibitory process does not depend upon prior activity other than the conducted impulse that sets it in operation. It may properly be said that the operation of the inhibitory process is revealed only by its contrary influence on excitation, for there is as yet no known potential sign that may be correlated with the threshold change evoked by the inhibitory process.

By virtue of their similarity of regression (compare Figs. 3 and 6) it seems that the inhibitory process should be identified as the functional opposite of residual facilitation. As yet there is no indication in experiment of an inhibitory counterpart of detonator action and, although this fact may indicate merely that the experimental conditions for its demonstration have not been satisfied, it is certainly unnecessary to assume such a counterpart in discussion of the known properties of inhibition.

Inhibition in two-neuron-arc systems is distributed strictly in accord with the requirements of reciprocal innervation (15), regardless of the anatomical location of the nuclei of the antagonist systems. Anatomical proximity of the nuclei belonging to the conditioning and testing systems, therefore, is not a factor in the production of inhibition. For example, direct inhibitory interaction obtains between the reflex pathways of quadriceps and biceps, muscles whose motor nuclei possess little segmental, or axial, overlap; whereas gastrocnemius and biceps, segmentally identical, and with their nuclei in close proximity in cross section, possess entirely independent two-neuron-arc reflex pathways (15). On any thinkable schema of inhibition it

would appear that the group I afferent fibers of quadriceps must approximate the reflex path of biceps, which they inhibit, more closely than do the comparable afferent fibers of gastrocnemius which are without action on the reflex path of biceps. Accordingly it is not reasonable to suppose that inhibitory interaction takes place outside the confines of motor nucleus of the inhibited muscle. It follows that there are two reasonable hypotheses: either inhibition results from the action of impulses in the terminal regions of some fibers upon the impulses in the terminal regions of other fibers, the net result being a decrease in the potency of the excitation delivered by the latter to the motoneurons (23), or inhibition results from the action of impulses in the terminal regions of fibers upon the somata of motoneurons. Since it is difficult to conceive of impulses affecting the terminations of fibers in a motor nucleus without at the same time influencing the motoneurons, the latter possibility merits the most careful and serious consideration; indeed, the two possibilities are not mutually exclusive.

*IX. Possible origin of brief summation periods.* Conditioning curves obtained by stimulation of dorsal roots, in contrast with those illustrated here, quite regularly and reproducibly exhibit brief initial periods of summation comparable to those described by Lorente de N6 (18). The only essential distinction between the two types of experiment, each involving stimulation of group I afferent fibers, lies in anatomical rather than functional selection of the afferent fibers for conditioning stimulation. Dorsal root stimulation of necessity produces volleys in antagonistic as well as allied reflex arcs. As a result of dorsal root stimulation, motoneurons are presented indiscriminately with the conditioning influences: detonator action, residual facilitation and inhibition. One may suppose that the latter two processes, being largely comparable except in direction and developing *pari passu* with strength of stimulation (11), would cancel each other, leaving detonator actions alone to determine the form of the interaction curve. On this interpretation, the brief facilitation periods obtained by the use of mixed volleys in dorsal roots—and presumably in tracts as well—define the summation period of the detonator process.

#### CONCLUSIONS AND SUMMARY

Facilitation and inhibition, by the direct actions of primary afferent fibers, of two-neuron-arc reflexes has been examined by experiment.

An afferent volley, in group I fibers arising in one head of a muscle, facilitates the action of its synergists and inhibits the action of its antagonists. Details of the distribution of these actions are presented elsewhere (15).

The temporal characteristics of facilitation and inhibition have been defined. Facilitation is maximal on the occasion of synchronous convergence of "conditioning" and "test" volleys, and decays exponentially along a curve falling to  $1/e$  in approximately 4.0 msec. Inhibition displays an incremental phase of approximately 0.5 msec. duration, thereafter decaying in the same manner as facilitation.

Reasons are given for supposing that the facilitation described here is

the expression of a process additional to the detonator action of earlier descriptions. Accordingly it may be called "residual facilitation."

The assumption of two excitatory events, detonator action and residual facilitation, makes no demand for elementary processes unknown in peripheral nerve. Their existence is predicted by the nerve-block model of synaptic transmission (18), only the significance of the latter, as far as the central nervous system is concerned, having remained in doubt in the absence of demonstration. The functional importance of residual facilitation now has been established.

According to present evidence, it is permissible to assume a correlation between residual facilitation and the "synaptic potential" of Eccles.

Residual facilitation and inhibition are regarded as functional opposites, they being similar in all known characteristics excepting direction.

Of many possible factors, three: detonator action, residual facilitation and inhibition, have received sufficient documentation to necessitate inclusion in theoretical consideration of the known properties of synaptic transmission.

Reason is given for supposing that the brief facilitation periods evident in appropriately designed experiments do, as had been supposed, measure the effective duration of the detonator action.

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# INTEGRATIVE PATTERN OF EXCITATION AND INHIBITION IN TWO-NEURON REFLEX ARCS

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THE PRECEDING paper presents an investigation of the elementary properties of the excitatory and inhibitory processes at the junctions of primary afferent fibers with the motor nuclei (9). Included are descriptions of residual facilitation and inhibition as events at such junctions additional to the earlier described detonator action (2, 10). The occurrence of inhibition, as a direct action, has been documented since 1941 (5); residual facilitation only now (3, 9) has proved to be quantitatively significant, although for some time the possibility of its existence has been considered (10). In the case of both actions it remains to demonstrate that the phenomena have significant relation to the integrative pattern of the spinal cord. The importance of such a demonstration can hardly be overemphasized, for in its absence an observed phenomenon may or may not have significance in reflex performance. A positive demonstration of lack of relation to integrative pattern, on the other hand, would indicate very strongly the essentially artifactual nature of an observed phenomenon. A fruitful technique for examining the functional significance of observed actions in the spinal cord (in this instance, residual facilitation and inhibition) is to determine their distribution among the several motor nuclei, when evoked by specified afferent volleys. This has been done, and the experiments to be described are representative of the results obtained.

The preparation used and the experimental arrangements were similar to those outlined in the preceding paper (9), with the exception that conditioning shocks sufficient to stimulate group I and group II afferent fibers were employed routinely. This was done in order to uncover the conditioning potentialities of the muscle afferent fibers. Eight combinations of conditioning and test volleys are to be considered.

## RESULTS

*I. Volleys in nerves of flexor muscles acting at the same joint.* Curve 1 of Figure 1 illustrates the conditioning effect upon a two-neuron-arc reflex, pertaining to biceps femoris posterior, of an afferent volley arising in the nerve to semitendinosus. These muscles are synergic flexors of the knee. It will be seen that facilitation of the test reflex begins with synchronous arrival of the conditioning and test volleys, and for brief intervals between the two volleys may be described by the curve typical of primary facilitation (9). The course of primary facilitation is interrupted by secondary facilitation, resulting from the stimulation of group II fibers, and representing flexor reflex activity.

*II. Volleys in nerves of flexor muscles acting at different joints.* Curve 2 of Figure 1 illustrates the result of conditioning the two-neuron-arc reflex

of a flexor muscle by afferent volleys deriving from flexor muscles of other nearby joints. The test stimulations were applied to the flexor fraction of the hamstring nerve (representing biceps femoris and semitendinosus, flexors of the knee), and conditioning stimulations were applied to the deep peroneal nerve (representing flexors of the foot). One notes in curve 2 of Figure 1 the

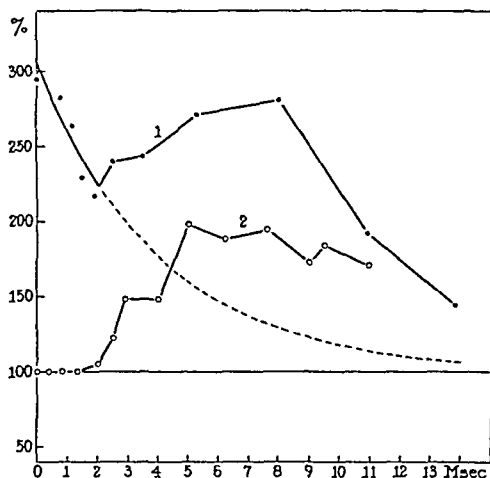


FIG. 1. Conditioning of two-neuron-arc reflexes by afferent volleys in group I and group II fibers of muscle nerves. In this and subsequent figures the conditioning effect on the test reflexes is expressed, on the ordinates, as the per cent of control amplitude achieved by the reflexes when conditioned. This is plotted as a function of time expressed on the abscissae, in msec. Solid line curves represent experimental points. The expected contribution to conditioning by the continued action of direct impulses (cf. 9) is expressed by the broken line extrapolations according to the exponential function proposed in the previous paper. In each case the interaction of afferent volleys arising in the nerves of muscles serving the same joint is indicated by dots, that of afferent volleys arising in the nerves of muscles serving neighboring joints is indicated by circles. Curve 1: Semitendinosus volleys conditioning biceps reflex. Curve 2: Deep peroneal volleys conditioning combined biceps-semitendinosus reflex.

of the flexor reflex. Analysis with the aid of the facilitation curve to be expected if group I fibers alone had been active (broken line) places the onset of inhibitory action within the second msec.

IV. *Volleys in nerves of extensor muscles that act at adjacent joints.* Curve 2 of Figure 2 charts the conditioning effect, upon the two-neuron-arc reflex of gastrocnemius (ankle extensor), of volleys arising among the afferent

the absence of any interaction between the systems until the conditioning volleys antecede the test volleys, at the spinal cord, by an interval of approximately 1.5 msec. Thereafter a period of facilitation obtains. This period of facilitation is comparable to the second period of facilitation in curve 1 of Figure 1 and, like it, is referable to the action through interneurons of group II impulses. The distinction between the two curves of Figure 1 lies in the presence (curve 1) or absence (curve 2) of facilitation attributable to the direct action of primary afferent fibers.

III. *Volleys in nerves of extensor muscles that act together at a joint.* Figure 2 illustrates (curve 1) the conditioning effect, upon the two-neuron-arc reflex of one head of gastrocnemius, of volleys arising in the nerve to the other head. The two heads of gastrocnemius naturally are synergic at one and the same joint. By the use of synchronous volleys the test reflex was increased to 485 per cent of its control amplitude, the experimental point not being indicated in the figure. The facilitation occurring at brief shock intervals, at greater intervals, is superseded by inhibition, the latter representing the inhibitory aspect

fibers of quadriceps (knee extensor). Inhibition, beginning at an interval of approximately 1.5 msec. separation of conditioning and test volleys at the cord, is quite evident, but there is no indication of interaction between the pathways of quadriceps and gastrocnemius at shorter intervals. Therefore the interaction of afferent volleys from extensor muscles, like those from flexor muscles, is characterized by the presence of direct effects—afferent fiber to motoneuron—if the muscles act as a common joint, or by the absence of direct effects if the muscles do not so act.

At this juncture it is well to correct an error made in 1944 (8, p. 12) while endeavoring to interpret direct inhibition on the basis of the information then available. It was thought, in view of the known interaction of group I afferent volleys stimulated in dorsal roots of various segments (5), that direct inhibition would account for the silent period shown by Denny-Brown (1) to occur in gastrocnemius as the reflex accompaniment of a tendon jerk in quadriceps. Experiment does not support the supposed relation since (i) there is no direct connection between quadriceps afferent fibers and gastrocnemius motor nucleus and (ii) the threshold for inhibition of gastrocnemius by stimulation of afferent fibers from quadriceps (as seen in Fig. 2, curve 2) accords with that of group II fibers.

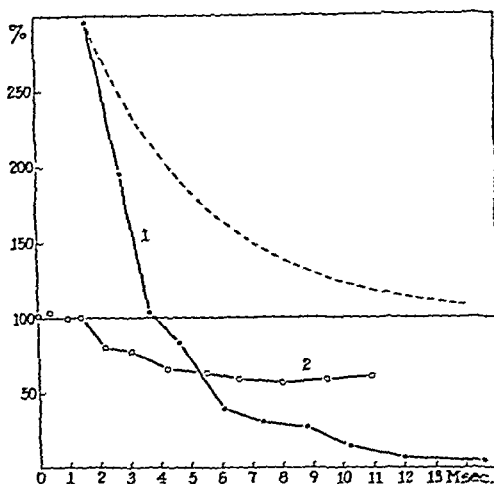


FIG. 2. Curve 1: Gastrocnemius afferent volleys conditioning gastrocnemius reflex. Curve 2: Quadriceps afferent volleys conditioning gastrocnemius reflex.

V. *Volleys in extensor nerve-flexor nerve sequence, the muscles of origin being antagonists at a given joint.* Curve 1 of Figure 3 illustrates the conditioning effect, on a two-neuron-arc reflex arising in the combined nerves of semitendinosus and biceps, and hence of knee-flexor origin, of volleys stimulated in the nerve of quadriceps (knee extensor). The initial effect of the conditioning volleys in this arrangement is inhibitory, the primary inhibition, after approximately 1.5 msec., being abrogated by facilitation of inter-nuncial and flexor reflex origin.

VI. *Volleys in extensor nerve-flexor nerve sequence, the muscles of origin serving neighboring joints.* By way of contrast with the preceding situation, it will be seen from Figure 3, curve 2 that afferent volleys arising in the nerves to gastrocnemius (ankle extensor) have no conditioning effect upon a two-neuron-arc reflex of biceps (knee flexor) origin unless the respective afferent volleys are separated by an interval of more than 1.5 msec. Prominent, however, is the secondary facilitation representing flexor reflex activity.

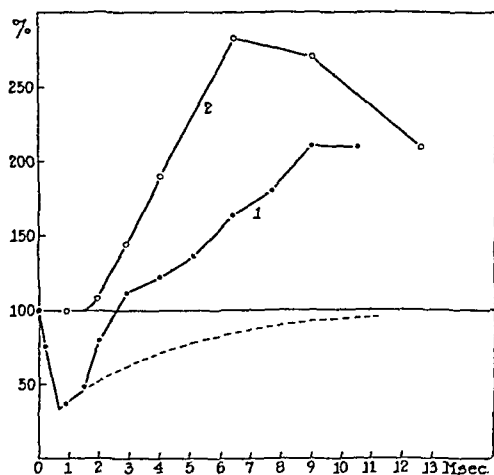


VII. Volleys in flexor nerve-extensor nerve sequence, the muscles of origin being antagonists at a given joint. Figure 4, curve 1 illustrates the conditioning of a two-neuron-arc reflex obtained by stimulation of the tibial nerve, by volleys arising in the deep peroneal nerve. The nerves stimulated represent muscles of extension and flexion of the foot respectively. Two successive periods of inhibition are apparent, the first beginning with synchronous arrival of the conditioning and test volleys, the second beginning when the test volleys follow the conditioning volleys by an interval slightly greater than 1.0 msec. The first period of inhibition represents direct action of primary afferent fibers, the second period representing the action of interneurons.

VIII. Volleys in flexor nerve-extensor nerve sequence, the muscles of origin serving adjacent joints. If the test reflex pertains to the ankle extensor, gastrocnemius, and the conditioning volleys have their origin in the knee flexor, biceps femoris, then but a single period of inhibition is realized (Fig. 4, curve 2). This period of inhibition is comparable to the second period of inhibition evidence in curve 1 of Figure 4, and is the result of internuncial action at the motoneurons.

*Conclusions relative to the distribution in motor nuclei of the direct actions of primary afferent fibers.*

FIG. 3. Curve 1: Quadriceps afferent volleys conditioning biceps-semitendinosus combined reflex. Curve 2: Gastrocnemius afferent volleys conditioning biceps reflex.



Considering together the experiments illustrated, it follows that the group I afferent fibers of one fraction of a muscle or muscle group, in addition to their powerful connections to the motoneurons of that fraction (6, 7), have direct excitatory connection with the remainder of the muscle or muscle group but not with muscles of like action serving neighboring joints, either proximally or distally situated in the limb. To parallel the excitatory connections, the group I afferent fibers of a given muscle group have direct inhibitory connection to the motor nuclei of antagonists that act as the same joint but not to the motor nuclei of functional opposites that serve neighboring joints. Thus a muscle, through two-neuron-arc reflex connections, is controlled by itself, its immediate synergists and its immediate antagonists. In turn, through like central connection, that muscle influences its immediate neighbors, synergists and antagonists.

Two-neuron-arc discharges are known to represent the myotatic reflexes (6, 7). In origin and distribution, inhibition by the direct action of primary afferent collaterals now is found to possess the characteristics of stretch-evoked inhibition of antagonists (4, 11). Direct facilitation of allied muscle

fractions is newly discovered (3, 9), but is seen to be distributed in strict accord with the requirements of reciprocal innervation. The absence of direct action on motoneurons pertaining to muscles of neighboring joints is consonant with the nature of myotatic reflex activity. In every instance the described actions of primary afferent fibers are expressions of reciprocal innervation. For these several reasons it is concluded that the actions described are of functional significance in the integrative pattern of the spinal cord and that they are concerned with the mediation of stretch-evoked, or myotatic, reflex performance. The muscles of a given joint being by direct reflex interconnection mutually dependent, and yet independent of other muscles at the myotatic level of postural performance, together constitute—along with the direct reflex paths that bind them—what may be called a myotatic unit.

Without the necessity of other than direct reflex connections, the myotatic unit exhibits within itself in full measure the elementary mechanism of reciprocal innervation. In the circumstances of the present experiments—that is, with the preparation in the spinal state—utilizing synchronous stimula-

tions so that the reflex discharges mimic phasic rather than static stretch responses, and with the possibility of feedback eliminated as the result of "opening" the reflex arcs for study, the flexor and extensor moieties of the myotatic unit possess essentially equal potentialities for reflex discharge, facilitation and inhibition. Therefore, it would seem that dominance of one moiety by the other arises largely as an expression of influences external to the myotatic unit. The reflex taxis of decerebrate rigidity provides the most obvious example.

Although the myotatic units are independent one from another in the central courses, it is possible for one unit to influence the next by action at the periphery. To cite a familiar example: quadriceps in active contraction acts to increase the distance between the origins and insertion of the two-joint gastrocnemius, and so subjects it to stretch. All things being equal, activity within the myotatic unit containing gastrocnemius would result. The same would not be true of quadriceps in relation to the single-joint soleus. Two-joint muscles, therefore, may be considered as bridges between individual myotatic units.

Before the distribution of inhibition within the myotatic unit was understood it was necessary to consider alternative possibilities: that it represented the silent period that appears in neighboring myotatic units (1, 8); that it represented the autogenous inhibition of the lengthening reaction;

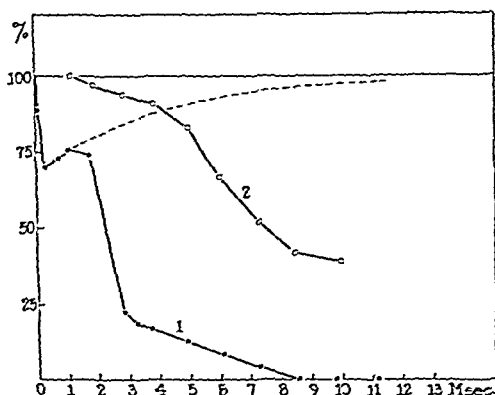


FIG. 4. Curve 1: Deep peroneal afferent volleys conditioning tibial nerve reflex. Curve 2: Biceps afferent volleys conditioning gastrocnemius reflex.

that it was an artifact. It is now clear that the silent period of Denny-Brown and autogenous inhibition are mediated by interneurons. Likewise, in view of its functional affinities, the possibility of direct inhibition, mediated by orthodromic volleys (5), being an artifact is very remote indeed.

### SUMMARY

An afferent volley arising in the nerve of a given muscle or muscle fraction has, by direct impingement upon motor nuclei, the following actions:—

1. If above threshold, it discharges motoneurons that supply that muscle or muscle fraction; otherwise, excitation is subliminal.

2. It facilitates the action of motoneurons that supply the muscle remainder, or synergists, at the same joint.

3. It inhibits the action of motoneurons that supply antagonists at the same joint.

The afferent volley in question, by direct action, neither excites nor inhibits motoneurons of muscles, flexor or extensor, that act at neighboring joints.

In every instance the actions described are in strict accord with the requirements of reciprocal innervation.

The origin and distribution of excitation and inhibition so evoked indicate the role they play in myotatic reflex performance.

The mutually dependent muscles of a joint, together with the direct reflex paths that bind them, may be considered as constituting a myotatic unit.

The myotatic units in the first instance are independent one from another. Two-joint muscles form peripheral bridges between adjacent myotatic units.

Without the necessity for other than direct reflex connections, the myotatic unit exhibits, complete within itself, the elementary mechanism of reciprocal innervation.

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# REFLEX CONTROL OF THE CILIARY MUSCLE

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REFRACTIVE changes in the mammalian eye are brought about mainly through contraction and relaxation of the ciliary muscle. This muscle comprises meridional and circular fibers. Radial components of the ciliary muscle have been postulated, but most investigators have failed to recognize radial fibers.

The efferent innervation of the ciliary muscle appears to be exclusively parasympathetic. The postganglionic nerve fibers in question arise in the ciliary ganglion. The preganglionic fibers through which this ganglion is connected with the central nervous system arise in the Edinger-Westphal nucleus, located in the mesencephalon, and traverse the oculomotor nerves. This mesencephalic autonomic center includes important central connections through which the reflex activity of the ciliary muscle is controlled.

In most of the histological studies bearing on the innervation of the intrinsic muscles of the eye, no attempts have been made to differentiate between the parasympathetic and the sympathetic components. The reported observations afford no adequate basis for the assumption that the ciliary muscle is innervated through both parasympathetic and sympathetic nerves. On the basis of observations on preparations of the eyes of cats—in some of which the sympathetic nerves, in others the parasympathetic, had undergone degeneration, following appropriate operative procedures—Stotler (7) and Clark (1) advanced the conclusion that the efferent innervation of the ciliary muscle is exclusively parasympathetic. The distribution of sympathetic nerve fibers within the ciliary body, according to their findings, is limited to the vascular bed.

Certain clinical and experimental data have been interpreted as incompatible with this point of view. For example, flattening of the lens in some degree associated with reflex dilatation of the pupil, observed repeatedly both in the experimental animals and in man, has been regarded as the result of contraction of radial components of the ciliary muscle elicited by impulses conducted through the sympathetic nerves. Among the more recent investigators, Cogan (2), Morgan, Olmsted and Watrous (4, 5, 6), and others have reported flattening of the lens in some degree in response to direct stimulation of the cervical sympathetic trunk. On the basis of such data and other clinical and experimental observations, various investigators, particularly Olmsted and his associates and Cogan, have advanced the conclusion that the sympathetic nerves play a role in the accommodation of the eye for distant vision.

The present investigation has been undertaken to obtain additional data regarding the reflex mechanisms through which the ciliary muscle is con-

trolled. Particular attention has been given to the role of the parasympathetic nerves in reflex responses tending toward hypermetropia elicited by peripheral stimulation and to the nature of the postganglionic fibers through which these reflexes are mediated.

#### EXPERIMENTAL PROCEDURES

The present investigation has involved experiments on cats and observations on human subjects. Approximately twenty young and adult cats have been used. The human subjects included adolescents and young adults ranging from 12 to 23 years of age. Some of the experiments on the cats have been designed to determine whether the reflex responses elicited by peripheral stimulation which result in reduction in the convexity of the lens are mediated through the sympathetic or the parasympathetic innervation of the eye. Others have been carried out to determine the nature of the postganglionic fibers involved in the reflex reactions through which reduction in the convexity of the lens is brought about.

All the experiments were carried out with the cats under nembutal anesthesia. Some were subjected to extirpation of the superior cervical sympathetic ganglion unilaterally several days in advance in order to insure functional degeneration of the sympathetic innervation of the eye before the other experimental procedures were initiated. Partial iridectomy was performed in advance of the other experimental procedures in some cats in order to facilitate measurement of the refractive changes in the eye without the use of pupillodilator drugs. Some also were subjected to extirpation of the adrenal glands before nerve stimulation was applied in order to obviate the possible effects on the intrinsic muscles of the eye of an increased output of adrenin.

For peripheral stimulation the faradic current was applied to the sciatic or another peripheral nerve and to the skin of the snout. The effects of such stimulation were observed in normal eyes, partially iridectomized eyes, and atropinized eyes and following intravenous administration of ergotoxine phosphate. Moderate faradic stimulation also was applied to the distal portion of the divided cervical sympathetic trunk and to the intracranial portion of the oculomotor nerve both before and after atropinization and following intravenous administration of ergotoxine phosphate.

The refractive changes produced in the eyes were measured by means of skiascopy. In order to obviate errors due to changes in the diameter of the pupil, the readings were regularly taken through a circular aperture 3 mm. in diameter in a metal disk. In some experiments a dome-shaped piece of metal with a centrally located circular aperture 3 mm. in diameter was used. This device, the periphery of which rested against the animal's head, was attached to the eye by means of four loops of thread drawn through the superficial layers of the sclera. By proper adjustment of these loops the aperture in the center of the metal was held in position over the center of the cornea and a few mm. from its surface. The eyes of the human subjects have been refracted while at rest and during mild peripheral stimulation. Those of the adolescents were refracted only after cycloplegia had been produced by instillation of homatropine into the conjunctival sac. Those of the young adults were untreated. Most of the skiascopic observations on the human subjects also were made through a 3 mm. aperture in a metal disk.

#### EXPERIMENTAL RESULTS

The results obtained in selected experiments on cats are outlined in the accompanying table (Table 1). Moderate faradic stimulation of the sciatic nerve regularly caused a refractive change of 1 diopter or over in the direction of hypermetropia both in the untreated and in the atropinized eye. The maximum change recorded due to sciatic nerve stimulation was 2.5 diopters. Comparable results were obtained both with the sympathetic nerves intact and following section of the sympathetic trunk in the cervical region or extirpation of the superior cervical sympathetic ganglion. Following intracranial section of the oculomotor nerve, but with the sympathetic trunk

Table 1. *Cats: nembutal anesthesia*

Drug administration	Operative procedure	Stimulation	Diopters as determined by skioscopy: Rt. eye	
			Before stimulation	After stimulation
None	Rt. cervical sympathetic trunk severed. Adrenal glands removed	Rt. cervical sympathetic trunk stim.		No change
		Sciatic nerve stim.	+4.	+6.5
	Both cervical sympathetic trunks severed. Adrenal glands removed	Skin of snout stim.	+4.5	+5.5
		Sciatic nerve stim.		No change
1% atropine alkaloid in oil instilled into conjunctival sac: 6 drops in 2 hr.	Rt. cervical sympathetic trunk severed. Adrenal glands removed	Rt. cervical sympathetic trunk stim.		No change
		Sciatic nerve stim.	+6.	+7.
	Rt. cervical sympathetic trunk severed and 3d cranial nerve exposed intracranially	Rt. 3d nerve stim. intracranially	+2.5	+3.5
1:500 ergotoxine phosphate into femoral vein: 1.0 cc. per kilo.	Partial iridectomy of rt. eye	None	+8.	
		Sciatic nerve stim.		No change
	Partial iridectomy of rt. eye. Rt. 3d cranial nerve exposed intracranially	Rt. 3d nerve stim. intracranially	+6.	+4.

\* The contralateral (left) eye of this cat served as a control. With the left 3d cranial nerve intact, the left cervical sympathetic trunk severed and the adrenal glands removed, sciatic stimulation resulted in a dioptric change from +6. to +7. in the control eye.

intact, sciatic stimulation elicited no dioptric change in the eye. The contralateral eye, with the oculomotor nerve intact, but deprived of its sympathetic innervation by section of the cervical sympathetic trunk, responded to the same stimulation with a refractive change of 1 diopter in the direction of

hypermetropia. Since the refractive response to stimulation of the sciatic nerve is not altered by interruption of the sympathetic innervation of the eye but is abolished by intracranial section of the oculomotor nerve, it appears to be mediated through the parasympathetic nerves. Since this reaction was not abolished by complete atropinization of the eye, it appears to involve the conduction of efferent impulses through adrenergic components of the short ciliary nerves. This conclusion is further supported by the failure of sciatic nerve stimulation to elicit a dioptric change in the eye following depression of the adrenergic fibers by the intravenous administration of ergotoxine phosphate (1 cc. per kilo of a 1:500 sol.).

Mild faradic stimulation of the skin of the snout resulted in refractive changes in the untreated and the atropinized eyes comparable to those produced by stimulation of the sciatic nerve. These responses also were mediated through the parasympathetic nerves, since they occurred alike while the sympathetic nerves were intact and following interruption of the cervical sympathetic trunk or extirpation of the superior cervical sympathetic ganglion.

Direct stimulation of the oculomotor nerve with the electrode applied to its intracranial portion regularly results in a marked refractive change in the direction of myopia in the untreated eye. Such stimulation following the intravenous administration of ergotoxine phosphate to depress the adrenergic nerves resulted in a change of 2 diopters or over in the same direction. When the eye was completely atropinized so that the cholinergic nerves were depressed, the same stimulation caused a refractive change of 1 diopter in the direction of hypermetropia. These results indicate that stimulation of the cholinergic components of the short ciliary nerves results in contraction of the ciliary muscle and increased convexity of the lens, whereas stimulation of the adrenergic components results in inhibition of the ciliary muscle and reduced convexity of the lens.

The observed results of direct stimulation of the cervical sympathetic trunk have not been unequivocal. In some experiments in which the electrode was applied to the intact vagosympathetic trunk, a change in the direction of hypermetropia was recorded which was approximately equal to that usually produced by peripheral nerve stimulation. This reaction probably involved reflex inhibition of the ciliary muscle elicited by visceral afferent impulses which resulted from efferent vagus stimulation. In experiments in which the cervical sympathetic trunk alone was severed and separated from the vagus nerve so that its peripheral portion could be stimulated without affecting the vagus (in animals from which the adrenals had been removed), stimulation of the sympathetic trunk which produced maximal dilatation of the pupil failed in most instances to produce a dioptric change in the eye.

Mild faradic stimulation applied to the skin of the forearm or the finger tips of the human subjects produced a dioptric change in the direction of hypermetropia. In the eyes of the adolescents, following cycloplegia, the

observed change usually did not exceed 0.25 diopter. In those of the young adults, which were untreated, the changes observed varied from 0.25 to 0.75 diopter. Of eight young adults whose eyes were refracted, two failed to show a dioptric change in response to the stimulation employed.

These responses appear almost insignificant in view of the greater magnitude of the dioptric changes in the eyes of the cats elicited by peripheral stimulation. It is significant, however, that in every instance the observed dioptric change tended in the direction of hypermetropia.

#### COMMENT

The results of the present series of experiments support the assumptions that the efferent innervation of the ciliary muscle is exclusively parasympathetic and that nerves of sympathetic origin effect direct contacts within the ciliary body only with the blood vessels. The innervation of the ciliary muscle, therefore, appears to be comparable to that of the sphincter muscle of the iris.

In these experiments, refractive changes in the eye in the direction of hypermetropia which have been elicited by peripheral stimulation have consistently been accompanied by dilatation of the pupil. Reduction in the convexity of the lens associated with reflex dilatation of the pupil also has been reported (2). The occurrence of both reduction in the convexity of the lens and dilatation of the pupil in response to peripheral stimulation, in eyes deprived of their sympathetic innervation, indicates that both reactions are mediated through the parasympathetic nerves. Since the cholinergic nerve fibers are depressed by atropine, the production of dioptric changes in the direction of hypermetropia in atropinized eyes by intracranial stimulation of the oculomotor nerve supports the assumption that inhibition of the ciliary muscle is brought about by impulses conducted from the ciliary ganglion through adrenergic fibers. This is in full accord with the conclusion, based on an earlier series of experiments (3), that reflex dilatation of the pupil in response to peripheral stimulation is an actively integrated reaction which involves the conduction of impulses from the ciliary ganglion to the circular muscle of the iris through adrenergic components of the short ciliary nerves.

The dioptric changes in the direction of hypermetropia elicited in the eyes of cats by mild faradic stimulation of the skin of the snout, and those elicited in human subjects by mild faradic stimulation, are of particular interest in relation to certain data advanced by Olmsted (5). He recorded in the eyes of a rabbit dioptric changes in the direction of hypermetropia accompanied by dilatation of the pupil, caused by "a sudden startling stimulus, such as a smart tap on the nose." He also reported momentary states of hypermetropia accompanied by dilatation of the pupil, in human subjects, caused by startling stimuli such as a sudden loud sound or an electric shock. Since these reactions were accompanied by other autonomic responses indicating sympathetic stimulation, such as cardiac acceleration and peripheral vaso-



constriction, he advanced the opinion that the momentary hypermetropia in man and the corresponding dioptric changes in the rabbit's eyes, caused by startling stimuli, were mediated through the sympathetic nerves.

The data set forth above are consistent with the view that dioptric changes in the direction of hypermetropia elicited by startling stimuli, like those elicited by peripheral nerve stimulation, are mediated through the parasympathetic innervation of the eye. In our experiments mild faradic stimulation of the skin of the cat's snout elicited dioptric changes in the eyes both before and after section of the cervical sympathetic trunks or extirpation of the superior cervical sympathetic ganglia which were comparable to those elicited by direct stimulation of the sciatic nerve. Since the reflex mechanisms through which these reactions are carried out remain intact following interruption of the sympathetic nerves to the eyes, they must involve the mesencephalic parasympathetic reflex center and a parasympathetic efferent pathway from that center.

Reflex dilatation of the pupil and dioptric changes in the direction of hypermetropia elicited through the parasympathetic nerves by peripheral nerve stimulation commonly are accompanied by vascular and other visceral responses which are mediated through sympathetic nerves. These facts are not incompatible since the eye reflexes in question involve efferent conduction of impulses through adrenergic postganglionic fibers. In the light of these findings, the normal reflex responses of the ciliary muscle, including those of accommodation, can be explained satisfactorily without assuming the existence of a sympathetic component in its innervation.

#### SUMMARY

In cats under nembutal anesthesia, faradic stimulation of a peripheral nerve or of the skin of the snout elicited a dioptric change in the direction of hypermetropia both before and after sympathetic denervation of the eye. Complete atropinization of the eye with resulting depression of cholinergic fibers did not abolish this response. It was abolished, however, when the oculomotor nerve was severed intracranially and when the adrenergic fibers were depressed by means of intravenous administration of ergotoxine phosphate.

Intracranial stimulation of the oculomotor nerve caused a dioptric change in the direction of myopia following depression of the adrenergic nerve fibers with ergotoxine phosphate, while identical stimulation of the oculomotor nerve following complete atropinization of the eye, with depression of cholinergic nerve fibers, resulted in a dioptric change in the direction of hypermetropia.

These results seem to indicate that reflex inhibition of the ciliary muscle is an actively integrated and controlled reaction mediated through the parasympathetic innervation of the eye which involves the efferent conduction of impulses from the ciliary ganglion to the ciliary muscle through adrenergic components of the short ciliary nerves.

In human subjects mild faradic stimulation of the skin of the forearm or of the finger tips elicited a dioptric change of small magnitude in the direction of hypermetropia in untreated eyes as well as during cyclopegia produced by instillation of homatropine into the conjunctival sac, with resultant depression of cholinergic fibers.

The reflex control of the ciliary muscle appears to be mediated exclusively through its parasympathetic innervation.

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# CORTICO-CORTICAL CONNECTIONS IN THE MONKEY WITH SPECIAL REFERENCE TO AREA 6

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## INTRODUCTION

ALL PREVIOUS investigations of cortico-cortical connections by physiological methods (4) have failed to reveal any homolateral afferent projections to area 6 of the precentral cortex. At the suggestion of Dr. Percival Bailey, the present study was undertaken in the effort to find homolateral cortico-cortical afferents to this area. Since a large portion of the cortex is buried in the depths of sulci, in certain instances these otherwise unavailable areas have been studied following removal of one of the banks of the sulcus. Thus, for the first time, the projection from certain portions of the cortex buried in sulci has been studied.

## METHOD

The method was that of physiological neuronography carried out on eight monkeys (*Macaca mulatta*), all under Dial<sup>†</sup> anesthesia. In those instances where the walls of various sulci were investigated, the opposite bank of the sulcus was first removed by careful subpial resection, care being taken that the vascular supply was not disturbed. To this end, hemostasis was secured only by fibrin foam. The medial surface of the hemisphere was reached by wide exposure of both hemispheres followed by clipping of the pial veins on the homolateral side as they enter the longitudinal sinus. The entire falx was then retracted to the opposite side, the homolateral hemisphere being allowed to fall laterally by posturing the animal. The orbital surface of the frontal lobe was exposed by extenteration of the orbit and removal of the roof and of the posterior wall. The technique of physiological neuronography is now well established and requires no restatement (2). Multiple electrode placements, 2 mm. apart, were systematically made throughout the whole of area 6, each electrode being identified on a coordinate grid. This will be referred to as the grid technique.

In recording the results the map of the macaque brain published by McCulloch (4) will be used except when noted (see Fig. 4).

## RESULTS

*Afferents to area 6a and 6b.* We will call area 6a that part of area 6 lying above and posterior to the arcuate sulcus and area 6b that part below and posterior to the inferior limb of the sulcus.

In many instances, firing into area 6a was quite restricted in its extent. For example, strychninization of area 46 in one animal resulted in firing only a 2 mm. square area, 4 mm. anterior to the superior precentral dimple; point-by-point exploration of the remainder of area 6a was completely

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† We wish to thank Ciba for placing the Dial at our disposal.

negative. In another animal, however, strychninization of the same area resulted in extensive firing of area 6a. Of the remainder of the precentral cortex only area 44 throughout its extent (Fig. 2) projects at area 6b. Multiple

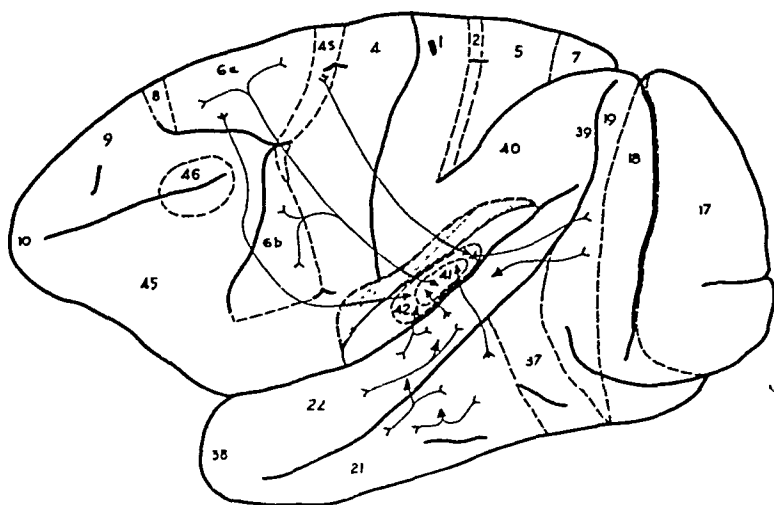


FIG. 1. Schematic representation of inter-areal connections from the temporal lobe.

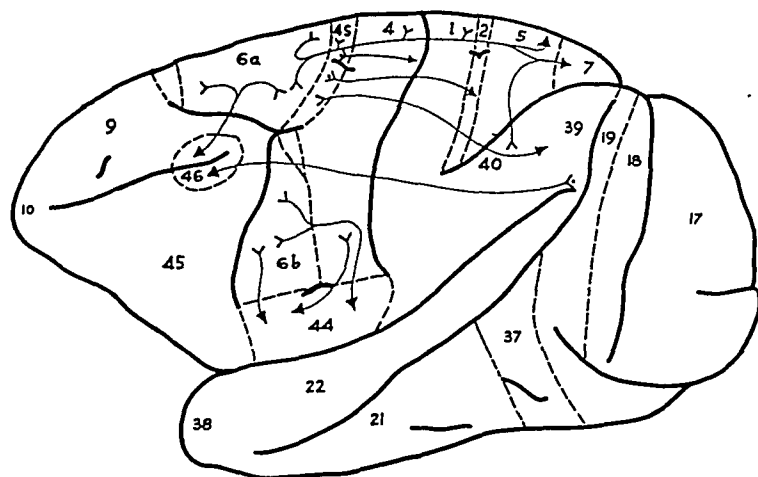


FIG. 2. Schematic representation of inter-areal connections of the lateral surface of the hemisphere.

strychninizations of the entire orbital surface (except for area 11) failed to fire into either area 6a or 6b.

In the temporal lobe, area 41 fired both 6a and 6b (Fig. 1). Area 41 was exposed by removal of the parietal operculum. Surrounding it is an area

(area 42) whose firing is more restricted in area 6a. In only two animals, area 42 appeared also as a narrow strip on the superior portion of the first temporal convolution. No firing into areas 6a or 6b was obtained from the remainder of the temporal lobe.

Of the cortex posterior to the central sulcus on the lateral surface of the hemisphere, only areas 5 and 7 projected into area 6a (Fig. 2). In all instances this was confined to the posterior third of area 6a.

Only that part of the medial surface of the hemisphere below and pos-

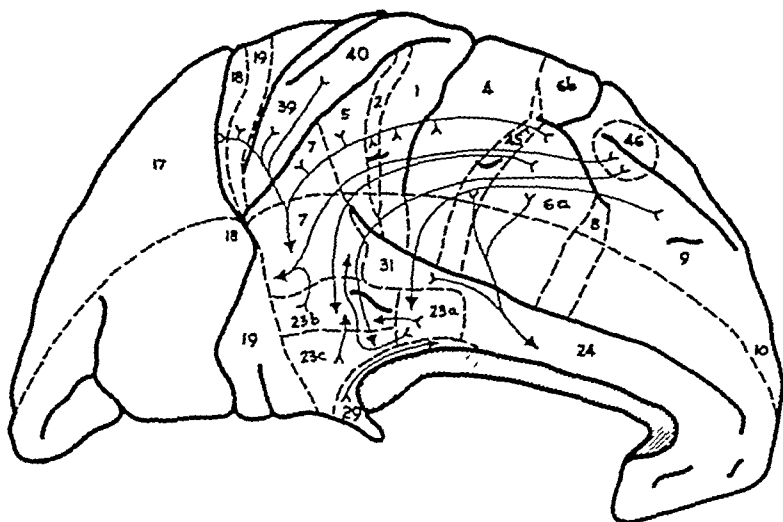


FIG. 3. Schematic representation of inter-areal connections from the physiologically distinguishable areas of the medial surface of the hemisphere.

terior to the cingulate sulcus has been investigated. On the basis of differential firing, this region has been divided into six areas lying anterior to area 19 (Fig. 3). Of these, only area 7 and area 23b fire into area 6a. One strychninization of the posterior part of area 24 fired a restricted portion of area 6a.

*Other connections of the medial cortex.* Of the five areas mentioned above, that one which we have found to have the richest cortico-cortical projections is area 7. On the medial surface area 7\* extends from the anterior border of area 19 to 2-3 mm. above the subparietal sulcus upward onto the lateral surface. In addition to its projection to area 6a, it has connections with areas 4s, 4, 1, 2, 5, 7, 39, 40, 19, 18, 23a and 23b. Only the posterior part of area 7 on the medial surface fires into area 46 (Fig. 3).

Directly below area 7 lies area 23b, which surrounds the subparietal sulcus, from area 19 posteriorly to area 23a anteriorly. Below, it is separated

\* Comparison of our map with that of McCulloch will show our area 7 extending forward to include part of McCulloch's area 31 (4).

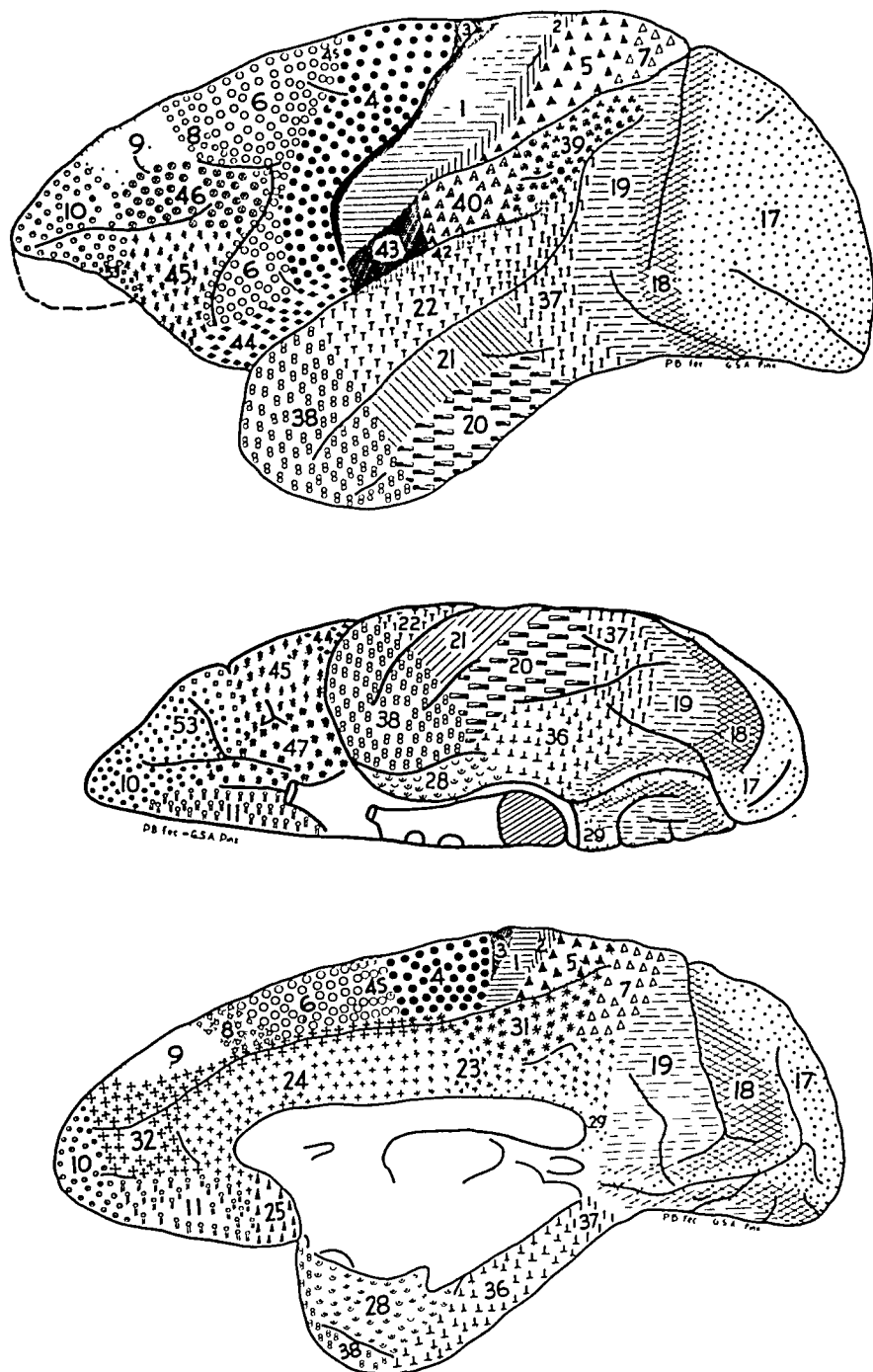


FIG. 4. Areas of cortex distinguishable by cytoarchitectonics or physiological neuronography in *Macaca mulatta*, taken from McCulloch (4).

from the corpus callosum by areas 23c and 29. In addition to its projections to area 6a, area 23b fired area 23a, but fired none of the areas on the lateral surface fired by area 7.

Area 23a lies anterior to areas 23b and 23c and is bounded below by area 29 and above by area 31. It fired only into area 9.

Areas 23c and 29 are two narrow strips separating the above areas from the corpus callosum. Area 23c projected only into area 46, whereas area 29 fired only itself.

Area 24, which comprises the anterior portion of the cingulate gyrus, projected to the anterior portion of area 6a, to area 4s and to area 31 along the lower lip of the posterior portion of the cingulate sulcus.

Area 19, on the medial surface, projected to area 18, on both the medial and lateral surfaces, as well as area 19 on the lateral hemispherical surface.

#### *Additional observations*

In addition to the projections which have already been mentioned, the following connections have been observed. Strychninization of area 46 on the lower wall of the principal sulcus (Fig. 2) caused firing into a minute zone of area 39 lying above the posterior extremity of the lateral fissure where it joins the superior temporal sulcus.

Only that part of area 44 anterior to the anterior subcentral dimple was found to fire into face 4. The posterior margin of leg 4, possibly the anterior margin of an area normally buried in the central sulcus, fired into area 4s, but only locally in the superior precentral dimple. Area 2 was also found to fire into 4s, as did area 39 and area 40. Areas 5 + 7 on the lateral surface fired areas 4s, 4, 1 and 40. (We have not been able to distinguish area 5 from 7 on the lateral surface and therefore refer to them as 5 + 7.)

Strychninization of the primary acoustic cortex (area 41), exposed by ablation of the parietal operculum, fired restrictedly into area 22 and also into area 21. Area 42, which surrounds area 41, fired more widely into area 22, but not into area 21; it fired locally into area 4s just below the superior precentral dimple. The most posterior part of area 42 fired into area 19 on the lateral surface, as did the posterior portion of area 22 on the convexity of the first temporal convolution. Unlike area 42, however, this part of area 22 did not fire into area 6. The remainder of the lateral surface of the first temporal convolution fired only itself in restricted areas, and locally into area 21. Strychninization of the upper wall of the second temporal sulcus resulted in restricted firing into nearby portions of area 22 on the convexity. Area 21 fired only itself.

#### DISCUSSION

*Afferents to area 6.* Afferent cortico-cortical connections to area 6 have been described by Mettler (5, 6, 7) and Milch (8) on the basis of degeneration studies with the Marchi method. The former made lesions in the superior temporal gyrus which invaded the external capsule and noted a uniform weak



degeneration of fine calibre fibers in area 6a (7). Lesions confined to the cortex of area 22 failed to cause such degeneration, although some degeneration in area 6b was observed. It has been here demonstrated that area 41 projects to areas 6a and 6b and undoubtedly the anatomical findings described above are due to involvement of fibers leaving the acoustic cortex. Extensive lesions of area 21, which Mettler claimed also caused degeneration in area 6, may well have injured these same pathways. No afferents from area 21 to areas 6a or 6b have been demonstrated by the strychnine method.

In two instances, strychninization of the superior portion of the lateral

*Table 1. Homolateral inter-areal connections of the lateral surface of the cerebral hemisphere*

Area strych.	12	9	46	10	44*	44†	4	1	2	5+7
Area recording										
9	—	—	—	—	—	0	—	—	—	0
46	—	—	—	—	—	0	—	—	—	0
10	—	—	—	—	—	0	—	—	—	—
8	—	—	0	—	—	0	—	—	—	—
6a	0	0	+	0	0	0	0	0	0	+
6b	0	0	0	0	+	+	0	0	0	0
44*	—	—	—	—	—	0	—	—	—	—
4s	—	—	0	—	0	0	+	—	+	+
4	—	—	0	—	+	0	—	—	+	+
1	—	—	—	—	—	0	—	—	0	+
2	—	—	—	—	0	—	—	—	—	0
5+7	—	—	—	—	—	0	—	—	—	+
39+40	—	—	+	—	—	0	—	—	0	+
19	—	—	—	—	—	—	—	—	—	0
22	—	—	—	—	—	0	—	—	—	—

\* denotes the portion of area 44 anterior to the anterior subcentral dimple.

† denotes the portion of area 44 between the anterior subcentral dimple and the lower end of the central sulcus.

+ indicates definite firing found.

0 indicates the area was investigated and no firing found.

Blank space indicates the area was not investigated.

surface of the first temporal convolution caused restricted firing of area 6a; this was undoubtedly due to a lateral extension of area 42, which normally lies completely buried in the depths of the lateral fissure.

Mettler (5) has also demonstrated afferent connections to area 6 from rather diffuse lesions of the frontal lobe. In all instances, these either involved a portion of area 6a itself, or they encroached upon area 46 which has here been demonstrated to project into area 6.

Milch (8) concluded that the postcentral gyrus and area 7 (Brodmann), which corresponds to our areas 39 and 40, were in intimate direct connection with area 6. Mettler (6) also found connections between the postcentral gyrus and area 6. We have found that whereas area 5+7 on the lateral surface projected to area 6a, areas 39 and 40 did not. The strychnine method

revealed connections from areas 5+7 but not from areas 1, 2, 39 or 40 to area 6a (Tables 1 and 2).

No afferents to areas 6a or 6b from the posterior aspect of the medial surface of the hemisphere have been previously described by either anatomical or physiological techniques (4). We have found very strong projections to area 6a from area 7 and more restricted projections from areas 23b and 24.

In many instances, firing into area 6a has been quite restricted in extent. This restricted firing, observed with the use of the grid technique, might well be missed with one pair of fixed electrodes placed at random in area 6a, which has been the usual technique of previous investigations (2).

*Medial surface of the parietal lobe.* On the superior portion of the medial surface of the parietal lobe, we have delimited an area 7 whose projections

Table 2. *Homolateral inter-areal connections of the lateral surface of the cerebral hemisphere*

Area strych.	39+40	19	18	17	41	42	22	21	38	orbital surface
Area recording										
9	0	—	—	—	—	—	0	—	—	—
46	0	—	—	—	—	—	0	—	—	—
8	—	—	—	—	0	0	0	—	—	—
6a	0	0	0	0	+	+	0	0	0	0
6b	0	0	0	0	+	0	0	0	0	0
4s	+	—	—	—	0	0	0	—	—	—
4	0	—	—	—	0	0	0	—	—	—
1	—	—	—	—	—	—	0	—	—	—
18	0	—	—	—	0	0	0	—	—	—
19	+	—	—	—	0	+	+	—	—	—
22	—	—	—	—	+	+	+	—	—	—
21	—	—	—	—	+	—	+	—	—	—

\* fired on the medial surface only.

+ indicates definite firing found.

0 indicates the area was investigated and no firing found.

Blank space indicates the area was not investigated.

are similar to those of areas 5+7 on the lateral surface (Tables 1 and 3). It is continuous with areas 5+7 anatomically; we have already noted that we cannot distinguish these two areas on the lateral surface by our findings. The only differences are minor; the posterior part of area 7 on the medial surface fired area 46 whereas areas 5+7 on the lateral surface did not. Table 3 gives the basis for the functional subdivision of area 23 into 23a, 23b and 23c.

*Lateral surface of the hemisphere.* An exhaustive study of the functional organization of the lateral surface of the hemisphere has not been repeated, but we have noted certain connections not previously described (4). Area 7 on the medial surface fired area 39, and area 46 was found to fire into a minute area, several millimeters in diameter, in the postero-inferior portion

degeneration of fine calibre fibers in area 6a (7). Lesions confined to the cortex of area 22 failed to cause such degeneration, although some degeneration in area 6b was observed. It has been here demonstrated that area 41 projects to areas 6a and 6b and undoubtedly the anatomical findings described above are due to involvement of fibers leaving the acoustic cortex. Extensive lesions of area 21, which Mettler claimed also caused degeneration in area 6, may well have injured these same pathways. No afferents from area 21 to areas 6a or 6b have been demonstrated by the strychnine method.

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46	—	—	—	—	—	0	—	—	—	0
10	—	—	—	—	—	0	—	—	—	—
8	—	—	0	—	—	0	—	—	—	—
6a	0	0	+	0	0	0	0	0	0	+
6b	0	0	0	0	+	+	0	0	0	0
44*	—	—	—	—	—	0	—	—	—	—
4s	—	—	0	—	0	0	+	—	+	+
4	—	—	0	—	+	0	—	—	+	+
1	—	—	—	—	—	0	—	—	0	+
2	—	—	—	—	0	—	—	—	—	0
5+7	—	—	—	—	—	0	—	—	—	+
39+40	—	—	+	—	—	0	—	—	0	+
19	—	—	—	—	—	—	—	—	—	0
22	—	—	—	—	—	0	—	—	—	—

\* denotes the portion of area 44 anterior to the anterior subcentral dimple.

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Area recording										
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46	0	—	—	—	—	—	0	—	—	—
8	—	—	—	—	0	0	0	—	—	—
6a	0	0	0	0	+	+	0	0	0	0
6b	0	0	0	0	+	0	0	0	0	0
4s	+	—	—	—	0	0	0	—	—	—
4	0	—	—	—	0	0	0	—	—	—
1	—	—	—	—	—	—	0	—	—	—
18	0	—	—	—	0	0	0	—	—	—
19	+	—	—	—	0	+	+	—	—	—
22	—	—	—	—	+	+	+	—	—	—
21	—	—	—	—	+	—	+	—	—	—

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10	—	—	—	—	—	0	—	—	—	—
8	—	—	0	—	—	0	—	—	—	—
6a	0	0	+	0	0	0	0	0	0	+
6b	0	0	0	0	+	+	0	0	0	0
44*	—	—	—	—	—	0	—	—	—	—
4s	—	—	0	—	0	0	+	—	+	+
4	—	—	0	—	+	0	—	—	+	+
1	—	—	—	—	—	0	—	—	0	+
2	—	—	—	—	0	—	—	—	—	0
5+7	—	—	—	—	—	0	—	—	—	+
39+40	—	—	+	—	—	0	—	—	0	+
19	—	—	—	—	—	—	—	—	—	0
22	—	—	—	—	—	0	—	—	—	—

\* denotes the portion of area 44 anterior to the anterior subcentral dimple.

† denotes the portion of area 44 between the anterior subcentral dimple and the lower end of the central sulcus.

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46	0	—	—	—	—	—	0	—	—	—
8	—	—	—	—	0	0	0	—	—	—
6a	0	0	0	0	+	+	0	0	0	0
6b	0	0	0	0	+	0	0	0	0	0
4s	+	—	—	—	0	0	0	—	—	—
4	0	—	—	—	0	0	0	—	—	—
1	—	—	—	—	—	—	0	—	—	—
18	0	—	—	—	0	0	0	—	—	—
19	+	—	—	—	0	+	+	—	—	—
22	—	—	—	—	+	+	+	—	—	—
21	—	—	—	—	+	—	+	—	—	—

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of area 39 (Fig. 2). This latter observation, together with those of Bailey *et al.* (1), establishes the existence of a two-way pathway between areas 46 and 39. These areas may represent merely the surface outcroppings of extensive areas buried in sulci.

The posterior part of McCulloch's area 44 (Fig. 2), lying on the precentral opercular lip of the lateral fissure, is similar in location to F3 of Dusser de Barenne *et al.* (3). It fired only into area 6b. The part of area 44 anterior to the anterior subcentral dimple likewise fired 6b as well as face 4; this cor-

Table 3. Homolateral inter-areal connections of the medial surface of the cerebral hemisphere

Area strychn.	24	23a	23b	23c	29	7	19
Area recording							
9	—	+	0	0	0	0	—
10	—	0	0	0	0	0	—
46	0	0	0	+	0	+	0
8	—	0	—	—	—	—	—
6a	+	0	+	0	0	+	—
6b	0	0	0	0	0	0	0
4s	+	0	—	—	—	+	0
4	0	0	0	0	0	+	0
1	—	—	0	—	—	+	0
2	—	—	—	—	—	+	—
5 + 7	—	—	—	—	0	+	0
39 + 40	—	0	0	—	0	+	—
19	—	0	0	—	—	+	—
18	—	0	0	—	—	+	+
17	—	—	—	—	—	0	—
7	—	—	—	—	0	—	—
23a	—	—	+	—	—	+	0
23b	—	—	—	—	—	+	—
23c	—	—	+	—	—	—	—
29	—	—	—	—	—	0	—
31	+	—	0	—	—	—	—
24	—	—	0	—	—	0	—

+ indicates definite firing found.

0 indicates the area was investigated and no firing found.

Blank space indicates the area was not investigated.

responds to Vogts' 6b  $\alpha$  and  $\beta$ . Even though these parts are difficult to distinguish histologically, they do not fire into each other, and their projections are not identical (Table 1).

From the posterior portion of the superior temporal gyrus, both areas 42 and 22 have fired area 19. However, area 42, buried in the lateral fissure, projects to area 6a, whereas area 22, on the convexity, does not—a fact which further distinguishes these two areas. No projections to area 19 have been previously described from these areas.

Area 19, like areas 2, 4s, 8 and 24, is a suppressor region. Of these, only 4s and 19 have been previously found to have afferent connections (4). We have found a number of such connections (Tables 1, 2, 3). The exact delimi-

tation of the suppressor areas is often difficult to establish, with the possible exception of areas 4s and 24. In most instances the firing into suppressor zones was exceedingly localized, which may account for previous failures to discover this firing.

These findings emphasize the fact that very localized projections exist which can only be demonstrated by a careful point-by-point search. Furthermore, they stress the necessity for investigating the two-thirds of the cortex which is buried in the sulci.

### CONCLUSIONS

1. Afferent cortical connections to area 6a have been described for the first time from areas 46 and 5+7 of the lateral hemispherical surface, areas 41 and 42 of the temporal lobe, and areas 7, 23b and 24 of the medial surface of the macaque brain by the method of physiological neuronography.

2. Additional observations on homolateral inter-areal connections have been made, including afferents to many of the suppressor regions.

We wish to thank Dr. Warren S. McCulloch and Dr. Percival Bailey for many helpful suggestions and criticisms in the interpretations of these results.

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# CENTRIFUGAL FUNCTIONAL DETERIORATION OF ASPHYXIATED MOTOR NERVE WITHIN THE NEURAL AXIS\*

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IN A RECENT paper (2) we showed that asphyxiated peripheral nerve undergoes centrifugal functional deterioration; *i.e.*, diminution and loss of excitability with respect to the somatic motor component occur first at the central end of the roots and spread distally along the roots and nerve trunk. This was demonstrated in spinal nerve in the cat. In the present experiments the behavior of asphyxiated cranial motor nerve in the cat was observed. Thorough study of the portion lying within the central nervous system proved that the nucleus is first affected. Diminution and loss of excitability progress thence distally along the nerve as it makes its way to the surface of the neuraxis and continues onward as the peripheral nerve.

## METHOD

The essential procedure was to apply electrodes to the nerve at different fixed points, observe the least electrical stimulus which at each point evoked a contraction of the appropriate muscles while the cat was breathing normally, and then clamp the trachea and follow the ensuing threshold changes.

The animals used weighed between 2.5 and 3.5 kg. Anesthesia was induced by administration of 50–60 mg. of chloralose per kg. body weight, which left reflex action brisk. The trachea was cannulated so that a good airway was assured till the time the trachea was clamped. The facial nerve was the test object in most experiments, though the hypoglossal was also used.

In each experiment on the facial nerve two unipolar electrodes were applied to different points on the intrabulbar portion of the nerve. They were inserted into the brain stem through small drill holes in the skull by means of the Horsley-Clarke instrument. The electrodes were clamped in the same electrode holder with their tips oriented so that they were related in space to each other and to the Horsley-Clarke reference axes precisely as were two predetermined points on the facial nerve. Thus, when one tip was at its desired location in the brain stem, the other tip was at its intended spot. Several combinations of points along the facial nerve were tested; in one instance one of the electrodes was inserted into the spinal tract of the fifth nerve for reflex activation of the seventh; and in several animals an additional electrode was placed on the zygomatic (zygomatico-orbital) branch of the facial nerve. In many cases the canthus reflex (blink elicited by tapping the inner canthus) also was under observation.

When the hypoglossal nerve was tested, one electrode was placed among hypoglossal fibers within the medulla, another on the peripheral nerve near the tongue. All electrode positions in the brain stem were later checked by histological examination. The animals were perfused with formalin while the electrodes were still in place so that the electrode positions were clearly discernible in the sections.

The stimulating electrodes were connected to a multiple throw switch and used in con-

\* Aided by a grant from The National Foundation for Infantile Paralysis to Northwestern University.

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junction with an anal dispersing electrode. The stimulator was that employed in the previous study of asphyxial nerve deterioration (2), producing shocks of an inverted saw-tooth wave form with the voltage, falling phase, and frequency controllable. In some instances 60 cycle alternating current was used for stimulation, with similar results. A frequency of one per second and a falling phase of 20 sigma were used in all our experiments from which data are presented. The electrical threshold at a stimulation point was the least voltage which elicited contraction of the orbicularis oculi muscle. It was necessary to enucleate the eyeball because otherwise the proptosis which accompanied the subsequently induced asphyxia interfered with the response. In the experiments dealing with the hypoglossal nerve the response for threshold testing was contraction of the intrinsic tongue muscles. When threshold determinations for different points were required close together in time,

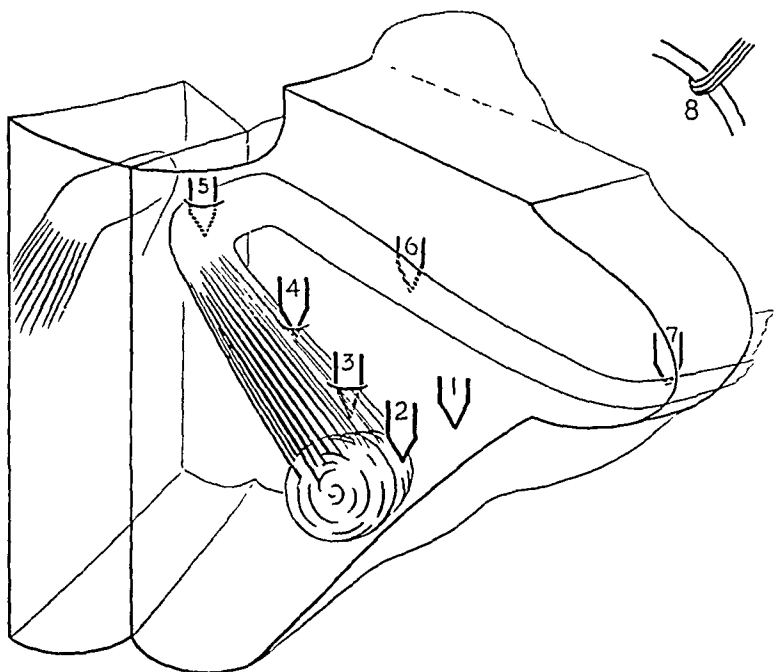


FIG. 1. Diagram of the intramedullary course of the facial nerve in the cat, seen from the caudal surface of a section 6 mm. thick. Electrode positions are indicated.

the most proximal point usually was tested first, followed immediately by respectively more distal ones.

After the electrodes were in place the control value of the thresholds for the points was determined periodically for at least 15 minutes to demonstrate constancy of excitability. Then the trachea was clamped and thresholds were observed in the ensuing asphyxia until deterioration progressed to complete or nearly complete inexcitability. Occasionally, maximal responses during asphyxia were observed visually and compared roughly with the control.

In some experiments the animal was heparinized and samples of arterial blood withdrawn periodically for pH determinations.

#### ELECTRODE POSITIONS ON FACIAL NERVE

Intramedullary course of the facial nerve is illustrated in Figure 1. This is a representation of a slice of the brain stem 6 mm. thick, cut in the

coronal Horsley-Clarke plane. It includes the rostral portion of the medulla oblongata and the caudal portion of the pons. The surface viewed, the caudal one, passes through the caudalmost end of the genu and about 1 mm. caudad from the caudalmost tip of the facial nucleus. The deep, rostral surface passes through the place of exit of the facial nerve from the brain stem. Most of the left side of the slice has been cut away. The facial nucleus has a caudo-rostral extent of 2 mm. The group of motoneuron perikarya which sends axons to the orbicularis oculi muscle occupies the dorsolateral part of the nucleus (5). The distance from this cell group to the internal genu of the facial nerve is 4 mm. The average length of fibers in the genu is 2 mm. Fibers leaving the genu extend laterally, ventrally, and somewhat rostrally for 7 mm. to make their exit from the brain stem. Thus, the length of the nerve fibers within the neuraxis is 13 mm. The nerve fibers from the brain stem surface to the orbicularis oculi muscle cover a distance of 67 mm., making the total fiber length approximately 80 mm. Between the nucleus and the internal genu the fibers course as scattered, small fasciculi. The fasciculi converge at the start of the genu, and from here on the fibers form a very compact tract.

The various electrode positions employed in the study of the facial nerve are shown in Figure 1. Electrode No. 1 is in the ventral part of the spinal fifth tract; No. 2 is on the lateral surface of the dorsolateral cell group of the facial nucleus; Nos. 3, 4, 5, 6 and 7 are among fasciculi or on the surface of the nerve tract between the facial nucleus and the exit of the facial nerve from the brain stem, and are respectively 1.5 mm., 2.5 mm., 4 mm., 8.5 mm. and 11.5 mm. distad from the nucleus along the fiber path; No. 8 is on the zygomatic branch of the facial nerve, 70 mm. distad from the nucleus. Electrode combinations used consisted of the following pairs: Nos. 1 and 6, 2 and 4, 3 and 5, 4 and 6, and 6 and 7. No. 8 was included in several experiments with various of these combinations.

## RESULTS

Figure 2 illustrates the degree of accuracy of localization of the electrodes. It is a low magnification photomicrograph of a section of the brain stem cut in the coronal Horsley-Clarke plane. The electrode tracks are those of Nos. 2 and 4. The tip of No. 2 was in contact with the dorsolateral cell group of the facial nucleus. The tip of No. 4 actually extended a fraction of a mm. deeper than seen in this section and rested against fasciculi between the nucleus and genu. The genu is cut at its caudal end and has received but a portion of its contributing fibers. Less than one mm. rostrally the genu is larger, more compact, and circumscribed in cross section. The placement of electrodes in other experiments was no less accurate than in this one, which was chosen for illustration because the plane of section passes through both electrode tracks.

Results were similar in all experiments. After clamping the trachea there was a transient reduction in threshold at each electrode followed by a rise

junction with an anal dispersing electrode. The stimulator was that employed in the previous study of asphyxial nerve deterioration (2), producing shocks of an inverted saw-tooth wave form with the voltage, falling phase, and frequency controllable. In some instances 60 cycle alternating current was used for stimulation, with similar results. A frequency of one per second and a falling phase of 20 sigma were used in all our experiments from which data are presented. The electrical threshold at a stimulation point was the least voltage which elicited contraction of the orbicularis oculi muscle. It was necessary to enucleate the eyeball because otherwise the proptosis which accompanied the subsequently induced asphyxia interfered with the response. In the experiments dealing with the hypoglossal nerve the response for threshold testing was contraction of the intrinsic tongue muscles. When threshold determinations for different points were required close together in time,

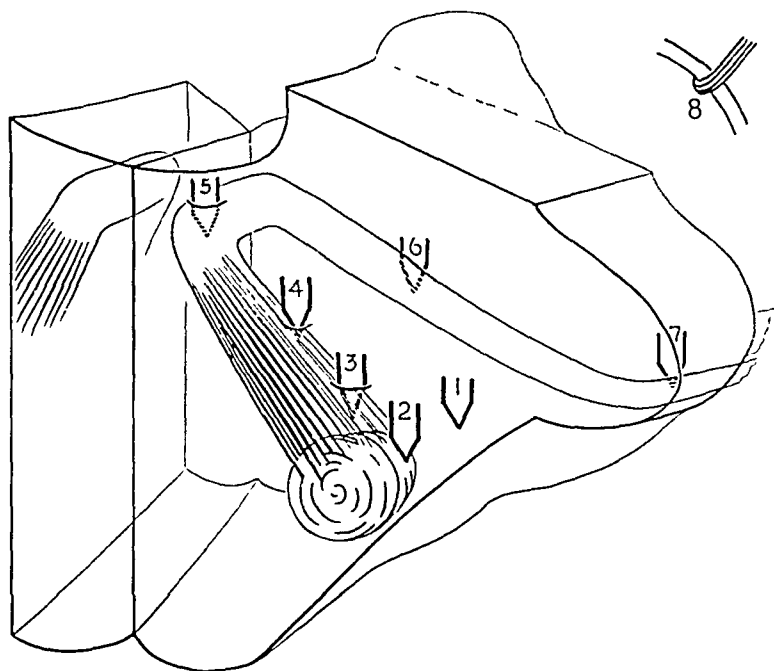


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#### ELECTRODE POSITIONS ON FACIAL NERVE

Intramedullary course of the facial nerve is illustrated in Figure 1. This is a representation of a slice of the brain stem 6 mm. thick, cut in the

points which exist beyond the limits of these charts. Portions of these curves in which there is a paucity of points are constructed to conform with results from other similar experiments.

The hypoglossal nerve behaved similarly. The nerve fibers underwent asphyxial deterioration much earlier within the brain stem than in the peripheral nerve.

The canthus reflex vanished in different experiments between one minute thirty seconds and three minutes fifteen seconds after tracheal occlusion. The most general time of disappearance was approximately two and one-half minutes.

Though no records of maximal responses were made, it was obvious that they were greatly diminished as thresholds rose.

### DISCUSSION

Since there was no experimentally introduced gradient in the environmental conditions along the nerve, it follows that regardless of immediate cause of the deterioration, its centrifugal course is a manifestation of a gradient that exists along the living nerve. This gradient we believe to be primarily functional with possibly a subtle structural counterpart, perhaps depending upon concentrations of chemical substances related to the constant proximo-distal flow of axoplasm for which some evidence has been presented (6, 7, 8).

The cause of functional deterioration of the nerves in these experiments is considered most likely to have been oxygen lack. Changes in pH were not implicated. The pH of arterial blood falls quickly after clamping the trachea, but after eight minutes, when the heart is beating exceedingly feebly, it is still in the neighborhood of 7.20. In experimentally lowered blood pH in cats, excitability of the facial nucleus remains unaltered above 6.90, increases but slightly at pH's as low as 6.60 (4).

The cause of the functional failure of the nerves, the spatial characteristics of the deterioration of fibers, and the basis of the gradient have been discussed at length previously (2).

It is apparent from Figure 3 that the time of onset of deterioration was not the same from experiment to experiment. Thus, deterioration in A and E set in earlier than in B and C, later than in D. There can be little doubt that there was a good deal of variation from one animal to another in the amount of oxygen and oxidizing reserve available to the tested nerve after clamping the trachea, and this inconsistency would certainly be adequate to account for any seeming incongruity of data from different experiments. When experiments are compared in which the canthus reflex disappeared at approximately the same time, the temporal course of threshold change is similar for similar points, and dissimilar points fall into proper temporal alignment from experiment to experiment.

It is obvious that, in the case of stimulation of the facial nucleus, we cannot specify for certain what structures in the nucleus we are stimulating—

which terminated in complete inexcitability. Invariably, the threshold began to rise first at the most centrally placed electrode, and began to rise at progressively more distal points at respectively longer intervals after the trachea was clamped. With passage of time the threshold rise was always furthest advanced at the most proximal point and less advanced at progres-

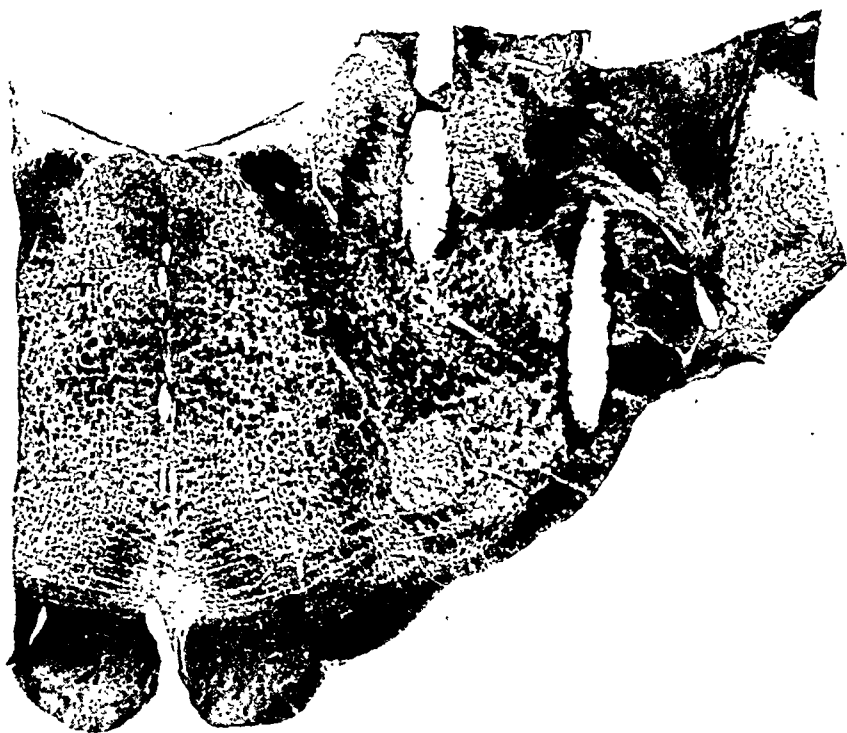


FIG. 2. Photomicrograph of a section through the brain stem of a cat showing tracks of electrodes Nos. 2 and 4. Weil's stain for myelin sheaths. Magnification  $-11.5\times$ .

sively more distal points until inexcitability or spread of current to other parts of the nerve intervened.

In Figure 3 are graphic representations of the results obtained with different electrode combinations. Each lettered division of the figure shows the threshold changes with time in a single experiment. The ordinates, on a logarithmic scale, are thresholds computed on the basis of the control value being equal to unity. The actual control thresholds for the various points in the different experiments fell in the range of 0.07 to 0.25 volt. The abscissae, on a uniform scale, are elapsed times after clamping the trachea. Threshold increment above four-fold is not shown because above this level the stimulus in some instances began to spread to spatially adjacent more distal sectors of the nerve. Lines directed upward beyond visible points are drawn through

this agnosticism might well be adopted in other experiments of diverse types on nuclear masses. One of us formerly believed, on a then perfectly legitimate basis, that threshold intensity at a nuclear electrode stimulated cells. In experiments on concussion (3) it was noted that with electrodes at positions (as described in the present paper) 2, 4 and 8, thresholds at 2 often rose abruptly in concussion while those at 4 and 8 did not. Asphyxiation was accompanied by threshold elevation first at 2, then at 4 and after a longer interval at 8 (1). The conclusion was that peripheral fibers withstood asphyxiation much better than central fibers, but that the latter were hardier than perikarya; and that (in the absence of consideration of a gradient) since the difference between nucleus and central fibers was discernible, the nuclear electrode stimulated perikarya. Though this reasoning is now invalid, the fact persists that concussion often diminishes excitability of some structure in the motor nucleus while usually leaving unaltered the axis-cylinder a short distance from it.

### SUMMARY

By means of placing stimulating electrodes along the intramedullary portion of the facial nerve and observing electrical thresholds before and after clamping the trachea, it has been shown that asphyxial deterioration of the nerve begins in the nucleus and extends progressively distalward with time. These results complement the previous finding of a proximo-distal deterioration gradient along the medial popliteal nerve and contributing ventral roots, and with it establish the existence of a gradient along the entire length of living motor nerve.

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dendritic endings, perikarya or axons—in the absence of detailed knowledge of respective thresholds. The nuclear threshold fell in the same range as thresholds at points along the intrabulbar course of the nerve. Hence it seems that the designated nuclear threshold either was threshold for the axons

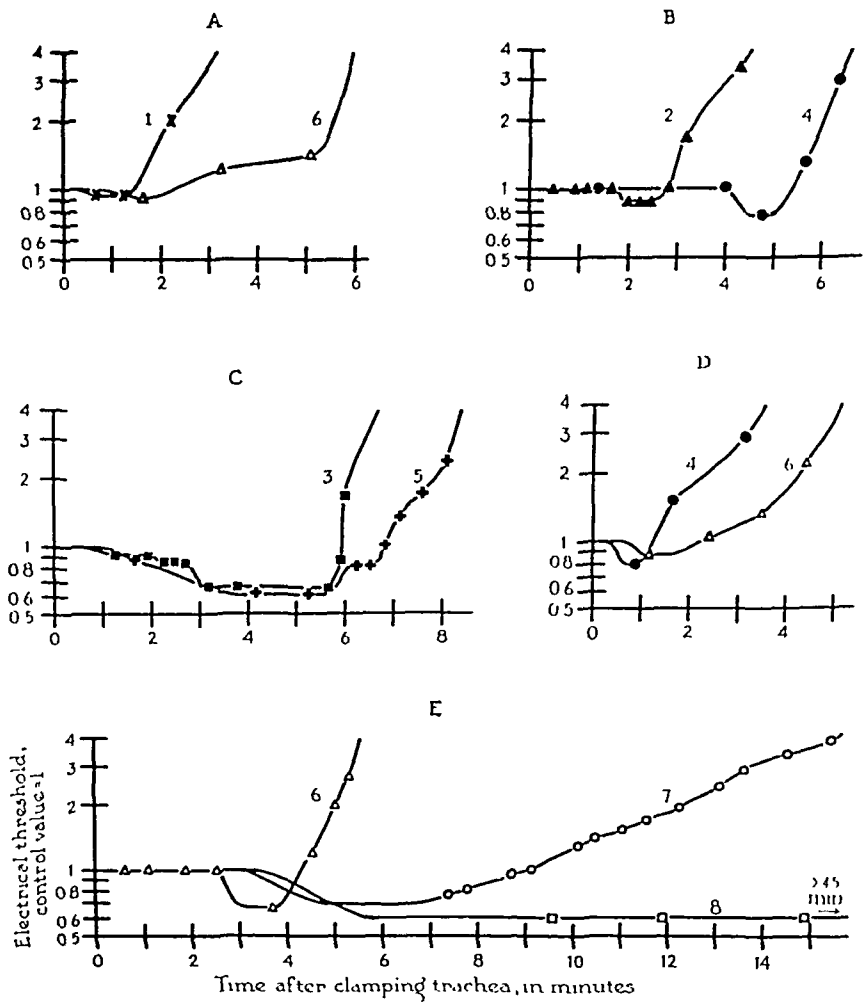


FIG. 3. Results of sample experiments in which different electrode combinations were employed. The numbers on the sets of points identify the electrodes, the positions of which are shown in Figure 1 and described in the text.

leaving the perikarya, or two or three hundredths of a volt lower. Since nuclear thresholds rose well above this level before onset of threshold increment at electrodes 3 and 4, we can safely conclude that the deterioration gradient involves the axon from its origin. Our experiments throw no light upon the behavior of the perikaryon in asphyxia. We wish to point out that

this agnosticism might well be adopted in other experiments of diverse types on nuclear masses. One of us formerly believed, on a then perfectly legitimate basis, that threshold intensity at a nuclear electrode stimulated cells. In experiments on concussion (3) it was noted that with electrodes at positions (as described in the present paper) 2, 4 and 8, thresholds at 2 often rose abruptly in concussion while those at 4 and 8 did not. Asphyxiation was accompanied by threshold elevation first at 2, then at 4 and after a longer interval at 8 (1). The conclusion was that peripheral fibers withstood asphyxiation much better than central fibers, but that the latter were hardier than perikarya; and that (in the absence of consideration of a gradient) since the difference between nucleus and central fibers was discernible, the nuclear electrode stimulated perikarya. Though this reasoning is now invalid, the fact persists that concussion often diminishes excitability of some structure in the motor nucleus while usually leaving unaltered the axis-cylinder a short distance from it.

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